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TO WILLIAM TOWNSEND PORTER

When physiological science in America was searching for a suitable medium for the publication of the increasing output of its laboratories and when no solution of the vexing problem seemed at hand, William Townsend Porter proposed the establishment of a new journal, to be called THE AMERICAN JOURNAL OF PHYSIOLOGY, and offered to undertake its administration. The American Physiological Society contributed its name and its moral support; Professor Porter took upon himself the editorial and the financial burdens. These he has borne through sixteen years and through the JOURNAL'S first thirty-three volumes. From its inception his ideals were high. He believed that a meritorious discovery may fail of appreciation because of the faulty manner in which it is announced to the world, and that an editor may be of service to an investigator. He believed that a scientific journal, the organ of a national science, should be characterised by scientific merit, rhetorical excellence, the prompt publication of its contributions, and typography and illustration that are pleasing to the eye. These ideals he has maintained. A rigid pursuit of ideals by one individual frequently arouses in others lack of appreciation, criticism, and opposition; and these he has received without complaint. Time and effort and sacrifice of personal considerations have been given by him without stint. In now laying down these burdens and generously transferring to the American Physiological Society his interests he has given over a journal that has an assured position of merit among journals of physiology and that has been one of the chief agencies in the unification of American physiology. For his unselfish labors Professor Porter deserves the thanks of American physiologists, and as an expression of this gratitude they gladly dedicate to him this volume.

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THE AMERICAN
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VOL. 37 APRIL 1, 1915 No. 1

STUDIES ON THE GROWTH OF MAN

1. The Pre- and Post-Natal Growth of Infants

T. BRAILSFORD ROBERTSON

From the Rudolph Spreckels Physiological Laboratory of the University of California

Received for publication January 10, 1915

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INTRODUCTION

It is a well-known fact that the growth of an animal in weight or linear dimensions does not proceed with uniformly retarded or accelerated velocity from the moment of its inception until the period of its completion. On the contrary, growth will at first take place relatively slowly, then more rapidly and then

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again more slowly, thus yielding an S-shaped curve of growth, and two or three of these sigmoid curves may be superimposed upon one another in the complete curve of growth of an animal. I have suggested that the period of growth covered by a *single* sigmoid curve should be termed a "growth cycle"[1] The extra-uterine growth of man consists of two growth cycles[2] and, as this article will show, a portion of a third, the inception of which occurs during the period of intra-uterine growth.

It has been shown by Read[3] that the intra-uterine growth of the guinea-pig consists of one whole growth-cycle and a portion of a second, birth occurring during the progress of the second growth-cycle. The point of junction of these cycles is a critical period in the growth of guinea-pigs. The juncture of the two cycles, at a period when growth is relatively slow, is not infrequently faulty, and as a consequence, it would appear, premature delivery of young, which are usually dead, occurs at this period much more frequently than at any other.

I have sought to ascertain whether or not a similar critical period occurs in the intra-uterine growth of infants. Through the courtesy of the Matron, Miss E. C. Sketheway, and of Dr. H. Gilbert, to whom I desire to express my very great indebtedness, I have had access to the extensive and admirably kept records of "The Queen's Home," a maternity hospital in Adelaide, South Australia.

2. The Pre-Natal Growth of South Australian Infants

I have sought to determine a portion of the pre-natal curve of growth from the weights of infants born at varying periods, somewhat prior to the normal period of gestation, and in order to obtain an estimate of the reliability of this method of procedure, I have also determined the weights of infants born somewhat

[1] T. Brailsford Robertson: Archiv f. Entwicklungsmechanik, 25 (1908), p. 581; 26 (1908), p. 108; 37 (1913), p. 497. Biologisches Zentralblatt, 30 (1910), p. 316; 33, (1913), p. 29. T. Brailsford Robertson and Hardolph Wasteneys, Archiv f. Entwicklungsmechanik, 37 (1913), p. 485.

[2] T. Brailsford Robertson: Arch. f. Entwicklungsmechanik, 25 (1908), p. 581.

[3] J. Marion Read: Arch. f. Entwicklungsmechanik, 35 (1912), p. 708.

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later than the normal period. Since, as we shall see, this latter curve overlies and is identical with the continuation of the normal-curve of extra-uterine growth backwards to the time of birth, we are entitled to infer that the curve determined by the weights of prematurely delivered, but otherwise normal children, represents the further continuation of the normal curve of growth, backwards into the intra-uterine period.

The data employed were exclusively obtained from "The Queen's Home," and cover the years 1909–1913. Patients, upon admission to this hospital, pay a small and frequently nominal fee, the fee being in many cases adjusted to the income of the patient. The patient secures admission through the recommendation of the doctor in charge of the case. Unmarried mothers are not admitted. The mothers therefore belong to the laboring and lower artisan classes.

The mother is usually admitted at a period as near as possible to that of labor and then remains in the hospital for 14 days after the birth of the infant. The infant is weighed, without clothing, at birth, and again upon discharge (13 to 15 days). Recently the practice has been instituted of also weighing the infant at 1 week (6 to 8 days after birth). The balance employed is accurate to within $\frac{1}{2}$ ounce.

The period of gestation, when ascertainable, is indicated on the patient's record, the date recorded being that of the onset of the last menstruation. In tabulating the data, only those (about two-thirds of the actually recorded data) were employed in which this data was accurately indicated. All infants stated to be suffering at birth from syphilitic infections, deformities, etc., and all infants which died within one week after delivery are excluded. The infants delivered by mothers who were suffering at the time of delivery or prior thereto from zymotic disease or other pathological conditions of a serious nature are also excluded.

The following (Table 1) were the results obtained for males, all infants born between 275 and 285 days after the onset of the last menstruation being tabulated as having been born at 280 days, all between 285 and 295 days as having been born at 290

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days, etc. Those born upon the limiting period separating two classes (e.g., 285 days) are included in both classes (e.g., 280 and 290).

A general tendency for increasing weight at birth to accompany increasing length of pregnancy is evident, but this becomes still more striking on *correcting* the above figures in the following way.

The *most probable* error in the estimation of the period of pregnancy is that of *one month*. Hence I assume that all periods

TABLE 1. MALES

(*Uncorrected Data*)

DAYS PREGNANCY	NUMBER OF INFANTS	AVERAGE WEIGHT IN OUNCES*
190	1	27
200	0	
210	1	54
220	0	
230	0	
240	1	123
250	2	117
260	22	119
270	38	121
280	79	127
290	78	130
300	16	139
310	9	138
320	3	123
330	1	152
340	0	

* I employ the ounce avoirdupois (1 ounce = 28.34 grammes) as the unit of weight because, in the first place, the weights of the infants were actually measured in terms of this unit and, in the second place, it is a unit of very convenient dimensions for this purpose.

of gestation which culminate in the delivery of infants weighing *less* than the uncorrected average for those born thirty days earlier, or *more* than the uncorrected average for those born thirty days later, are erroneously estimated and that the infants delivered in these cases should be excluded on account of this uncertainty in the estimation of the period of gestation. In this way we also probably exclude the majority of crypto-syphilitic infants born at the later periods of gestation.

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The following (Table 2) are the *corrected* results for males, excluding those born before 260 or after 310 days as being two few in number and possibly representing pathological or erroneously estimated gestations.

The data for the 280-day infants were not corrected for three reasons. *Firstly*, because the 280-days group is the group nearest to the mean, the mean period of gestation for male infants (cf. below) being 282.5 days. *Secondly*, because the average weight of the 280-day infants (127 ounces) is the nearest to the average weight of *all* of the infants (127.3 ounces). *Thirdly*, because errors in the estimation of this period would obviously tend to cancel one another, being equally probably plus or minus.

TABLE 2. MALES
(*Corrected Data*)

DAYS PREGNANCY	NUMBER OF INFANTS	NUMBER OF INFANTS EXCLUDED	AVERAGE WEIGHT IN OUNCES
260	16	6	111
270	34	4	117
280	79	0	127
290	60	18	137
300	13	3	145
310	6	3	146

The following are the corresponding figures for females—

TABLE 3. FEMALES
(*Uncorrected Data*)

DAYS PREGNANCY	NUMBER OF INFANTS	AVERAGE WEIGHT IN OUNCES
190	1	39
200	2	59
210	0	
220	1	83
230	2	90
240	3	95
250	6	97
260	10	111
270	32	113
280	80	117
290	86	125
300	31	129
310	14	130
320	3	127
330	1	134
340	0	

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TABLE 4. FEMALES

(*Corrected Data*)

DAYS PREGNANCY	NUMBER OF INFANTS	NUMBER OF INFANTS EXCLUDED	AVERAGE WEIGHT IN OUNCES
250	6	0	97
260	8	2	105
270	27	5	108
280	80	0	120
290	70	16	130
300	25	6	133*
310	11	3	138

* Excluding one infant weighing 184 ounces at birth which died within a fortnight of delivery.

As in the case of the male infants and for similar reasons, the data for the 280-day group are not corrected.

3. The Mean Period of Gestation for South Australian Infants

From the data recorded in Tables 2 and 4, it is possible to construct a curve displaying the intra-uterine growth of infants from the 250th day of gestation until the 310th day. In order to determine which portion of this curve represents *normally* intra-uterine growth and which portion represents normally extra-uterine growth it is necessary to determine with some exactitude the *mean period of gestation* for South Australian infants.

In attempting to determine this period, we might employ the average of all the different periods of gestation enumerated in Table 1 or 3, but in so doing, as we have seen, we should probably include some marked deviations from the true average, which represent departures from the mean period of gestation, which are not purely fortuitous and intrinsic in origin, but due to the intrusion of definite extrinsic variables such as pathological conditions of the mother or infant or large errors (1 month) in the estimation of the observed periods.

We might employ some arbitrary criteria for the exclusion of extreme deviations, as I have done in selecting the restricted

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data enumerated in Tables 2 and 4. It should be noted, however, that the criterion employed in selecting these data is one which does not depend upon the magnitude of the period itself but upon the magnitude of another variable, namely the weight of the infant after delivery. For the purpose of obtaining the most probably correct estimates of the weights of infants delivered after varying periods of gestation, this procedure is justified, since by this means not only erroneously estimated periods of gestation are probably eliminated from the data, but also infants which, although born at correctly estimated periods, deviate so widely from the mean weight corresponding to those periods as to justify the suspicion that their development has been of an abnormal character. Abnormal development of the infant may frequently, but by no means necessarily, influence the length of the period of gestation. Consequently we should not be justified in excluding observed periods of gestation not differing too extremely from the mean on the sole ground of the super- or subnormal weight of the infant delivered.

We are therefore led to inquire what procedure we can employ, depending solely upon the magnitudes of the observed and apparently normal periods of gestation, which will enable us to exclude from the data enumerated in Tables 1 and 3 those of which the deviations from the mean are more probably due to extrinsic than to intrinsic variables, i.e., which are probably due to determinate but undetected large errors of estimation or pathological conditions.

Such a procedure, determined solely by the observed magnitude and not dependent upon any *a priori* considerations added thereto, is afforded by Chauvenet's criterion for the rejection of extreme variates,[4] which is widely employed in statistical investigations and physical measurements which involve a large number of determinations.[5] This criterion is evaluated in the following manner:

Referring to Table 1, we observe that out of a total of 251

[4] W. Chauvenet: A Manual of Spherical and Practical Astronomy, 5th ed. 1891, 2d vol., p. 558.

[5] Cf. C. B. Davenport: Statistical Methods, 2d ed., New York, 1904, p. 12.

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male infants, one was born at 190 days, one at 210 days, one at 240 days, two were born at 250 days, and so forth, the average period of gestation for all of these infants being 281.8 days.

We now determine the deviation of each of the observed periods of gestation from the above average. Thus the deviation of the 190-day period is 91.8 days, that of the 330-day period is 48.2 days and so forth. Square each of these deviations, multiply each of these squares by the number of individuals displaying the deviation in question and add the products together. Thus Table 1 yields:

$91.8^2 \times 1 + 71.8^2 \times 1 + 41.8^2 \times 1 + 31.8^2 \times 2 + 21.8^2 \times 22 + 11.8^2 \times 38 + 1.8^2 \times 79 + 8.2^2 \times 78 + 18.2^2 \times 16 + 28.2^2 \times 9 + 38.2^2 \times 3 + 48.2^2 \times 1 = 74319.$

Divide this sum by the total number of infants (= 251) and take the square root of this quotient. The value thus obtained, 17.2, is the *standard deviation* of the period of gestation for male infants. The standard deviation is a measure of the variability of any quantity provided that quantity only varies accidentally, that is to say, in accordance with the laws of probability indifferently in excess and in defect of its mean value.[6]

When a series of magnitudes which deviate fortuitously from the mean are tabulated in classes, as we have tabulated periods of gestation in Tables 1 and 3, we find that those classes (in Table 1, the 280- and 290-day classes) which lie nearest in magnitude to the mean contain the greatest number of examples, i.e., exhibit the greatest "frequency." If we plot the frequencies of the classes vertically, employing their deviations from the mean as abscissae, we obtain, as is well-known, the "probability-curve."

$$y = \frac{n}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}}$$

in which n is the total number of "variates" (in this instance 251, the total number of infants), σ is the "standard deviation"

[6] Cf. C. B. Davenport: Statistical Methods, 2d ed., New York, 1904, p. 15. G. Udny Yule: An Introduction to the Theory of Statistics, 2d ed., London, 1912, chapter 8.

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determined in the manner outlined above, y and x are the ordinate and abscissa respectively, and e is the base of the Napierian logarithms.

The general form of this curve is familiar. The majority of the variates lie close in magnitude to the mean, and therefore the greater part of the area enclosed between the curve and the axis of the abscissae lies close to the maximum ordinate, i.e., that expressing the number of variates exactly equal in magnitude to the mean. The curve slopes away upon either side of the mean, at first rapidly and then more slowly. The abscissa of the point of inflexion is σ, the standard deviation.

Assuming that the observed deviations of the experimental magnitude (in the particular instance in hand, the period of gestation) are for the most part purely fortuitous and therefore lie upon or near to the probability-curve, and having determined the "standard deviation" of the observed magnitudes, we can now proceed to determine which, if any, of the experimental deviations from the mean are probably not fortuitous in the following way:

Let x_1 be the magnitude of a given deviation, a expressed in terms of the standard deviation, so that $\frac{a}{\sigma} = x_1$, then the integral:

$$\varphi(\sigma x_1) = \frac{2}{\sigma\sqrt{2\pi}} \int_0^{\sigma x_1} e^{-\frac{x^2}{2\sigma^2}} dx$$

expresses the proportion of variates of which the deviation from the mean is *less* than a. If we multiply this by n, the total number of variates, we obtain $n\varphi(\sigma x_1)$ which is the actual number of variates of which the deviation from the mean is less than a. Substracting this from n we have:

$$n - n\varphi(\sigma x_1) = n[1 - \varphi(\sigma x_1)]$$

which is the number of deviations which must be expected to be *greater* than a. If now this quantity is less than $\frac{1}{2}$ it will follow that a deviation of magnitude a has a greater probability against it than for it, and we may infer that among a limited number of

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purely fortuitous deviations it would not occur. Such a deviation from the mean we may therefore reject as being improbably fortuitous. The criterion for rejection is therefore obtained from the equation:

$$\varphi(\sigma x_1) = \frac{2n-1}{2n}$$

We have now to find the value of σx_1 which corresponds to an area of the probability-curve equaling $\frac{2n-1}{2n}$ where n is the total number of observations, in this instance 251. We can ascertain the value of x_1 by referring to tables of probability-integrals (such as, for example, Table IV in Davenport's "Statistical Methods" referred to above).

We have $\frac{2 \times 251 - 1}{2 \times 251} = 0.99801$. One-half of this area lies on either side of the mean, the tables of probability integrals give the values of x_1 corresponding to given areas on *one* side of the mean. We therefore divide the above area by 2, obtaining the area 0.49900. The table of probability-integrals shows that the value of x_1 which corresponds to this area is 3.09. Hence the limit of allowable deviation from the mean is given by:

$$a = \sigma x_1 = 17.2 \times 3.09 = 53.$$

This is therefore the maximum deviation from the mean period of gestation which may be expected to occur among 251 observations provided all of the observed deviations are fortuitous. Any period of gestation greater than $282 + 53 = 335$ days or less than $282 - 53 = 229$ days may therefore be eliminated from the observations as being probably attributable to the intrusion of extrinsic factors. Referring again to Table 1, we see that the 190- and 210-day periods may be rejected in computing the average magnitude of the period of gestation for males.

But in computing this maximum allowable deviation we began by assuming (in determining the "standard deviation") that the observed deviations from the mean were all fortuitous in origin. Nevertheless we have found that two of the observed

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deviations were probably not fortuitous, but due to the intrusion of some extrinsic undetected variable into the system of varia' les which normally determine the length of the period of gestation. This renders a new application of Chauvenet's criterion necessary, in the carrying out of which we exclude these two observations and treat the remainder of the observed periods as the basis of a fresh estimate of the "standard deviation," the area of the probability-curve corresponding to the extreme allowable deviation and so forth, until we finally, by successive applications of Chauvenet's criterion, eliminate all the observations of which the deviations from the mean (corrected by the omission of these values) are too great to be merely fortuitous, and obtain a series of estimates of the period of gestation, all of which may legitimately be regarded as representing fortuitous deviations from a fixed average value.

Treating the data enumerated in Table 1 in this manner, we find that the *first* application of Chauvenet's criterion yields the limiting classes 229–335 days. The infants born at 190 and 210 days are therefore excluded. The *second* application of Chauvenet's criterion yields the limiting classes 242–322 days. The infants born at 240 and 330 days are therefore excluded. The *third* application of Chauvenet's criterion yields the limiting classes 243–321 days and leads to no further exclusions. We conclude therefore that with four exceptions, namely, the 190, 210, 240, and 330 day periods, all of the periods of gestation enumerated in Table 1 may be regarded as fortuitous departures from the true mean.

The number (N) of observed periods with the exception of those excluded by the above process is 247. The standard deviation (σ) for these periods is 12.7. The average of these periods is 282.5 days. The "probable error" of this estimate is given by $\pm 0.6745 \frac{\sigma}{\sqrt{N}} = \pm 0.55$, which means that the chances are even (1 to 1) that the true value of the mean period of gestation for males lies between 281.95 and 283.05 days.[7]

[7] Cf. C. B. Davenport: loc. cit. p. 15.

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Applying the same methods of computation to the data for female infants enumerated in Table 3 we find that the *first* application of Chauvenet's criterion yields the limiting classes 228–338 days. The infants born at 190, 200, and 220 days are therefore excluded. The *second* application of Chauvenet's criterion yields the limiting classes 241–329 days. The infants born at 330 days are therefore excluded. The *third* application of Chauvenet's criterion yields the limiting classes 241–327 days and leads to no further exclusions. We conclude therefore that with 7 exceptions, comprising the 190, 200, 220, and 330 day periods, all of the periods of gestation enumerated in Table 3 may be regarded as fortuitous departures from the true mean.

The number (N) of observed periods with the exception of those excluded by the above process is 264. The standard deviation (σ) for these periods is 13.8. The average of these periods is 284.5 days. The "probable error" of this estimate is $\pm$ 0.57. The chances are therefore even that the true period of gestation for female infants lies between 283.93 and 285.07 days.

From these results it appears that the mean period of gestation for female infants is longer than that for male infants. The probability of the truth of this conclusion is the inverse of the probability that either of the above estimates, namely, that of the period of gestation for male infants or that of the period of gestation for female infants, differs from the true mean by four times the "probable error" of the estimate of either mean, which is the extent of the divergency of the two estimates. Hence the probability of the truth of the conclusion that the period of gestation is longer for female infants than for male infants is 142 to 1.[8]

It should be noted that the ordinary method of estimating the probable period of gestation, namely, that of adding seven days to the date of the onset of the last menstruation and subtracting three calendar months from that date in the following year, yields periods which vary in length between 280 and 283 days.

[8] Cf. C. B. Davenport, loc. cit,, p. 14.

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4. The Mean Weight at Delivery of Normally Delivered South Australian Infants

We have seen that with the exclusion of a small number of extreme and probably pathological or erroneously estimated deviations, the mean period of gestation for South Australian male infants is 282.5 days, while that for female infants is 284.5 days. We may assume, therefore, that with the same exclusions the mean weight of the infants at birth represents their weight at this period of their development, namely, at 282.5 days for males and 284.5 days for females.

The average weight at birth of all of the male infants, exclusive of those born at periods of gestation which are rejected by Chauvenet's criterion, is 127.3 ounces. On referring to Table 2, it will be seen that this lies between the weight of male infants born at 280 days and that of male infants born at 290 days, considerably closer to the weight of the former than that of the latter group.

The average weight at birth of all of the female infants, exclusive of those born at periods of gestation which are rejected by Chauvenet's criterion, is 121.2 ounces. On referring to Table 4, it will be seen that this lies between the weight of female infants born at 280 days and that of female infants born at 290 days, again considerably closer to the weight of the former than to that of the latter group.

The "standard deviation" of the weight at birth is 18.2 ounces for male and 17.6 ounces for female infants. The standard deviation is a measure of the *absolute variability* of a quantity. The *percentage variability* is yielded by the ratio of the standard deviation to the mean multiplied by one hundred.[9] Thus the percentage variability of the weight of South Australian male infants at birth is

$$\frac{18.2}{127.3} \times 100 = 14.3 \text{ per cent}$$

[9] Karl Pearson: Phil. Trans. Roy. Soc. London, 187 A, (1896), p. 253. Cf. C. B. Davenport, loc. cit., p. 16.

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while that of the weight of South Australian female infants at birth is

$$\frac{17.6}{121.2} \times 100 = 14.5 \text{ per cent}$$

These figures mean that out of any group of normally delivered male infants selected by chance 68.27 per cent or approximately two-thirds will weigh within 14.3 per cent of the mean weight, while out of any group of normally delivered female infants selected by chance two-thirds will weigh within 14.5 per cent of the mean weight.

The British Anthropometric Committee[10] reports that the average weight at birth of 451 male infants born in London and Edinburgh is 113.6 ounces, while that of 466 female infants is 110.4 ounces. According to Pearson,[11] the mean weight at birth of 1000 male infants born in London (Lambeth Lying-in Hospital) is 116.8 ounces with a variability of 15.7 per cent, while that of 1000 female infants is 113.2 ounces, with a variability of 14.2 per cent. Through the courtesy of Dr. Smallwood Savage and of Dr. Elsie M. Humpherson, to whom I desire to express my great indebtedness, I have been furnished with the weights of one hundred male and one hundred female infants at birth, chosen without selection from the records of normal full-term deliveries in the Maternity Hospital of Birmingham, England. From these data I find that the average weight of male infants at birth in Birmingham is 114.9 ounces with a variability of 13.2 per cent, while the average weight of female infants at birth is 113.5 ounces, with a variability of 12.5 per cent.

From these figures, a striking fact emerges, namely, that South Australian infants weigh from 8 to 10 ounces more at birth than infants born in Great Britain. This fact is remarkable because, according to an article on the "People of Australia," contributed by G. H. Knibbs, Commonwealth Statistician, to the *Federal Handbook on Australia* issued by the British Association for the Advancement of Science in 1914, "The Australian

[10] Report of the British Assoc. for the Adv. of Science, 1883, p. 285.
[11] Karl Pearson: Proc. Roy. Soc. London, 66 (1899), p. 23.

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people, with regard to racial constitution, are virtually British, as the following figures from the last census show, and it may be added that the descendants of other European races disclose but small differentiation from their fellow citizens of British origin. The percentages of the principle races represented are as follows:

"Australian born 82.90 per cent; natives of United Kingdom 13.37; of New Zealand 0.72; of Germany 0.75; of China 0.47; of Scandinavia 0.33; of all other places 1.46; that is to say, at the date of the census, 1911, no less than 97 per cent had been born either in Australasia or in the United Kingdom.

"The evolution of the Australian people, therefore, may be regarded as that of the British people under changed climatic, social, and economic conditions."

The results observed cannot be due to any racial selection among the immigrants from the British Isles who have given rise to the population of Australia, for in the first place there is no evidence that such selection has occurred to any greater extent than it has for example, in London, of which city the very great increase in population during the past century has been contributed by all parts of the British Isles, and in the second place there is no evidence of the existence of a race in the British Isles which is in any noteworthy degree superior in physical dimensions to the average inhabitant of England.[12] We can only infer, therefore, that the superior weight of the Australian infant at birth is attributable to the factors enumerated by the Commonwealth Statistician, namely, the change in climatic, social, and economic conditions. The climate is much less rigorous than that of England, food is cheaper in proportion to income or, when of a like price, better in quality, and the social and economic conditions are so far an improvement upon those prevailing in England that whereas women of the laboring and lower artisan classes in England have frequently to supplement the family income by their own exertions, those of the corresponding classes in Australia as a rule confine their physical activities to the management of their households and families, a condition of

[12] Cf. Brit. Anthropometric Committee's report, Report of the British Assoc. for the Adv. of Science, 1883, p. 253.

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affairs which, quite apart from the possible direct effects of physical labor upon the mother, must have important indirect effects in ameliorating the nutritional and hygienic conditions within the household. These conditions may be conceived to have an appreciable pre-natal effect upon the growth of children since Pinard has shown that the rest and improved nutrition of hospital life causes a notable increase in the average weight of infants delivered by working mothers who spend a part or a whole of the period of pregnancy in a lying-in hospital[13] and Prochownik has shown[14] that the size of a child at delivery may be reduced by restricting the diet of the mother.

Not only are South Australian infants heavier at birth than infants born in the British Isles, but they are also from 5 to 6 ounces heavier at birth than infants of English descent born in the Eastern United States, for according to Bowditch[15] the average weight of Anglo-American male infants at birth is 120.8 ounces, that of Anglo-American female infants 115.7 ounces. The Anglo-American infant is therefore intermediate as regards weight at birth between the Australian infant and the British infant. This very clearly corresponds with the character of the social and economic conditions prevailing in these three countries. It would appear, therefore, that the mean weight of infants of the same race at birth is a very sensitive criterion of the social and economic environment in which they are born.

It will be observed that the *variability* of the weight of South Australian infants at birth is very nearly the same as that of infants born in London or in Birmingham, with this difference, that whereas Pearson finds for London infants that the variability of males is greater than that of females, and the figures above cited for Birmingham infants exhibit a somewhat smaller excess of variability in males, South Australian male infants would appear not to be more variable at birth than females.

[13] Cited after G. Newman: Infant Mortality, London, 1906, p. 81.

[14] Cited after G. Newman: loc. cit., p. 84.

[15] H. P. Bowditch: Eighth Annual Report, State Board of Health, Massachusetts, 1877. Cited after Report of the British Assn. for the Adv. of Science, 1879, p. 200.

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The various data concerning the weights of British infants at birth in different localities are exhibited in tabular form below:

TABLE 5

PLACE OF BIRTH	SEX	NUMBER WEIGHED	MEAN WEIGHT AT BIRTH IN OUNCES	VARIABILITY
				per cent
London and Edinburgh (British Anthropometric Committee)	Male	451	113.6	
	Female	466	110.4	
London (Pearson)	Male	1000	116.8	15.7
	Female	1000	113.2	14.2
Birmingham	Male	100	114.9	13.2
	Female	100	113.5	12.5
Eastern United States (Bowditch)	Male	100	120.8	
	Female	100	115.7	
Adelaide, South Australia.	Male	247	127.3	14.3
	Female	264	121.2	14.5

5. The Post-Natal Growth of South Australian Infants

The object of this investigation being primarily to determine the relationship of the curve of pre-natal growth, determined from the weight of normal infants somewhat prematurely delivered, to the curve of post-natal growth, it was necessary for the attainment of this object to determine the post-natal curve of growth for South Australian infants of the class (laboring and lower artisan) from which the data for weight at birth were obtained. These data I was enabled to obtain through the kindness of Miss A. Hornabrook (Hon. Secretary of the Adelaide School for Mothers), Miss H. A. Stirling (member of the Committee), and Nurse Clara Webb (Superintendent) by whom the data in the possession of the "Adelaide School for Mothers" were placed at my disposal and to whom I desire to express my very great indebtedness.

The "Adelaide School for Mothers Institute" offers instruction to mothers regarding the feeding and care of their infants at a nominal charge. At frequent intervals the Registrar of Births for the State of South Australia reports to the Institute the births which have occurred in its neighborhood, and a nurse in the em-

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ploy of the Institute then calls upon those mothers who, being known to be more or less needy in circumstances, are likely to have insufficient medical advice, and proffers them the services of the Institute. The infants are voluntarily brought to the Institute by the mothers upon certain days when the nurse is in attendance. The infants, without clothing, are weighed by the nurse and the weight and date are noted upon a card bearing the infant's name. Other data of importance, such as the nature of the infant's food, illnesses, etc., are also noted upon the card and these records are kept on file.

The infants which are brought to the Institute therefore belong to the laboring and lower artisan classes. They include a large proportion of sickly infants, since mothers who appreciate the benefits accruing from the advice and help they have received advise friends, and especially friends with ailing infants, to bring their children to the Institute.

The following data (Tables 6 to 9) are computed from the records of this Institute accumulated in the years 1910–1913 up to but not including those of July 21, 1913. All data concerning infants suffering from definite ailments or requiring medical attendance are excluded. When infants which were otherwise normal contracted zymotic diseases, such as mumps, measles, etc., the weights recorded prior to the decrease in weight immediately preceding or accompanying recognition of the disease are included in the data, subsequent weighings being rejected. In cases where no loss of weight had occurred at the time of recognition of the disease, weights preceding recognition of the disease were included, weighings subsequent to this being excluded. Data concerning twins were excluded.

The data are obtained from repeated weighings at irregular intervals of 159 "normal" infants, classified as follows:

Breast-fed males	63	
Bottle-fed males	27	Total males 90
Breast-fed females	43	Total females 69
Bottle-fed females	26	

The "breast-fed" classes include all infants stated to have been breast-fed, irrespective of the period during which they were so

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fed. Infants are considered to have been bottle-fed when there is no record of breast-feeding upon their cards. Hence some of the bottle-fed infants which were first brought to the School for Mothers during the latter months of their first year were probably breast-fed during some period prior to the date when they first came under observation.

The weights are grouped into periods of 30 days. All weights recorded between the 15th and 45th days succeeding births are regarded as weights at 30 days and so forth. When more than one weighing was recorded for one infant in one such 30-day period, the different weighings are averaged, fractions of an ounce less than $\frac{1}{2}$ being regarded as zero, while fractions of an ounce equal to or greater than $\frac{1}{2}$ are regarded as 1 ounce. Weighings falling exactly upon the limiting date separating two periods (e.g., exactly upon the 45th day) are placed in both classes (e.g., the 30-day and the 60-day classes).

The balance employed for weighing was accurate to 1 ounce. The following are the summarized data (Tables 6 and 7).

The superiority of the breast-fed child, which has so frequently been commented upon by numerous observers, is here again strikingly revealed. On comparing the above data with similar data for English children, such as Sir George Newman's standard

TABLE 6. MALES

AGE IN MONTHS OF 30 DAYS	BREAST-FED		BOTTLE-FED	
	No. infants weighed	Average weight in ounces	No. infants weighed	Average weight in ounces
1	20	155	5	117
2	27	187	14	141
3	30	206	11	169
4	26	224	9	193
5	24	254	9	226
6	23	270	8	242
7	25	287	3	267
8	22	300	3	329
9	20	311	8	280
10	11	326	6	298
11	10	333	7	322
12	6	330	5	335

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TABLE 7. FEMALES

AGE IN MONTHS OF 30 DAYS	BREAST-FED		BOTTLE-FED	
	No. infants weighed	Average weight in ounces	No. infants weighed	Average weight in ounces
1	12	153	3	120
2	26	168	11	137
3	23	188	10	156
4	20	209	11	179
5	21	224	10	184
6	17	253	11	198
7	15	263	8	212
8	9	270	8	239
9	8	300	6	259
10	6	315	7	252
11	6	335	7	265
12	5	345	7	288

growth-curve,[16] it is evident that the superiority of the Australian child at birth is maintained in varying proportion throughout the first year of post-natal life. This remains the case even when allowance has been made for the fact, which I will show in a subsequent publication, that Sir George Newman's standard is somewhat too low.

6. The Formulation of the Relationship Between Age and Weight in the Post-Natal Curve of Growth for South Australian Infants

In seeking to determine whether or to what extent the curve of pre-natal growth determined from the weights of prematurely delivered infants represents the continuation of the post-natal curve of growth, we might proceed by simply drawing a curve through the points determined upon the post-natal curve, and continuing it backwards from birth estimate approximately the degree of resemblance of this graphically constructed curve to the pre-natal curve of growth. Such a method would yield only a very approximate and indefinite comparison, however.

[16] G. Newman: Infant Mortality, London, 1906. Cf. also H. W. Pooler, Sixth Annual Report of the Birmingham Infants' Health Society, 1913.

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A much more reliable comparison may be made by fitting some algebraic interpolation-formula to the post-natal curve. Such a formula will yield, by inserting in it varying values of the time coördinate, not only weights interpolated between those ascertained by measurement, but also values exterpolated therefrom and continuing the curve in either direction (i.e., pre-natally or subsequently to the twelfth month of post-natal growth). Such continuations may be relied upon, if the interpolation-curve be so chosen as to fit the observations closely, to represent the true continuation of the curve of growth, provided only that the exterpolation is not too extreme, that is, provided we do not seek to extend the observation-curve too far.

A large number of well-known interpolation-formulae, which are frequently employed in physical and statistical investigations are at our disposal for this purpose. We might, for example, express the weight x of an infant in terms of ascending powers of t, its age, thus:

$$x = a + bt + ct^2 + dt^3 + \ldots\ldots$$

where a, b, c, d, etc., are constants[17] and it would only be a question, as in other physical measurements, of employing a sufficient number of terms and constants to obtain an equation expressing to any desired degree of accuracy the observed magnitudes.

I have, however, shown in previous communications[18] that the relationship between weight and time for any single growth-cycle is the same as that which subsists between the extent of transformation and the time in an autocatalysed chemical reaction, that is to say, a reaction one of the products of which accelerates it. The formula expressing this relationship is:

$$\log \frac{x}{A - x} = K\,(t - t_1)$$

[17] C. Henry et L. Bastien: Comptes rendus de l'Association Francaise pour l'avancement des Sciences, 1904. P. Enriques, Biologisches Centralbl., 29 (1909), p. 331. T. Brailsford Robertson, Ibid, 30 (1910) p. 316.

[18] T. Brailsford Robertson: Arch. f. Entwicklungsmechanik, 25 (1908), p. 581; 26 (1908), p. 108.

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where A is a constant (= the maximum weight attained by the particular growth-cycle under consideration), t_1 is a constant (= time at which the growth-cycle is half-completed), K is a constant, and x and t are the weight and time respectively.

We may therefore employ this formula as a means of exterpolating from (continuing) the observed curve of growth in preference to any of the other interpolation-curves at our disposal. It should be noted, however, that the results of the exterpolation are quite independent of any particular deductions concerning the actual nature of the growth-process. I have concluded from the form of the curve representing a growth-cycle and from other data[19] that the process of growth represents the progress of a self-accelerated chemical reaction. Should this conclusion prove invalid, however, the conclusions reached by employing the curve of autocatalysis as a means of exterpolating from the observed curve of growth will not be invalidated, for the form of the exterpolated curve, for moderate exterpolations would be substantially the same whatever the form of the algebraic expression employed to represent the observed data. On the other hand, the convenient form of the algebraic expression for the curve of autocatalysis and the small number of constants involved present obvious advantages over more unwieldy formulae which might yield an equally faithful reproduction of the observed data.

We shall only employ, for the purpose of exterpolation, the data for breast-fed infants, since these may safely be presumed to be the most "normal" data available.

In fitting the curve of autocatalysis to the results for breast-fed males (Table 6) we proceed as follows[20] to obtain approximate values of A, K and t_1, in the equation

$$\log \frac{x}{A - x} = K\,(t - t_1).$$

Representing graphically in the usual manner the curve of

[19] T. Brailsford Robertson: loc. cit.; also Arch. f. Entwicklungsmech., 37 (1913), p. 497.

[20] T. Brailsford Robertson: Arch. f. Entwicklungsmech., 25 (1908), p. 581.

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growth of South Australian infants during their first post-natal year it is at once evident that the end of the first year represents approximately the conclusion of a sigmoid curve. In this region the growth-curve for man enters, as is well-known, upon a "plateau" or period of relatively slow growth such as characterises the conclusion of a growth-cycle. The value of A for this cycle is therefore, probably, greater, but not very much greater than the value of x (= weight in ounces) at 12 months. Hence, as a first approximation, we may take the value of A for South Australian males (cf. Table 6) as 334 ounces. The value of t_1 is the value of t when $x = \frac{1}{2}A = 167$; this is the point of inflexion of the curve, when from being convex it becomes concave to the time-axis. We observe (Table 6) that at one month after birth $x = 155$ ounces, while at two months after birth $x = 187$ ounces. The point of inflexion occurs at a value of t, therefore, lying between one and two months. Let y be the fraction of a month in excess of one at which the point of inflexion occurs. Then to a first approximation:

$$y = \frac{167 - 155}{187 - 155} = 0.375$$

Hence t_1 equals, approximately, 1.375 months, reckoning from birth.

The value of x *at birth* ($t = 0$) is probably the most accurate, since 247 infants were weighed in determining this value for males. Putting the above values of A and t_1 in the equation $\log_{10} \frac{x}{A - x} = K(t - t_1)$, and putting $t = 0$ and $x = 127$ (= weight at birth) we obtain $K = 0.154$.

The equation $\log_{10} \frac{x}{A - x} = K(t - t_1)$ may be written

$1/K \log_{10} \frac{x}{A - x} + t_1 = t$. Inserting the above approximate values of the constants, we obtain:

$$6.5 \log_{10} \frac{x}{334 - x} + 1.375 = t.$$

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in which t is the time which has elapsed since birth, and x is the weight of the infant.

We now proceed to correct these arbitrary and approximate values of the constants from all of the observations by the method of least squares as follows:[21]

Designating the arbitrarily chosen constants by the symbols a, b, and c, so that a $\log_{10} \frac{x}{b-x} + c = t$, let the *most probable* values of these constants be designated, respectively, $1/K$, A and t_1.

$$\begin{aligned} \text{Then } 1/K &= a + \alpha = 6.5 + \alpha \\ A &= b + \beta = 334 + \beta \\ t_1 &= c + \gamma = 1.375 + \gamma \end{aligned}$$

where α, β and γ are small corrections the *most probable* values of which are now to be determined from all of the observations cited in Table 6.

Expanding the function:

$$\varphi = 1/K \log \frac{x}{A-x} + t_1 - t.$$

by Taylor's Theorem, inserting the above values for $1/K\ (=a+\alpha)$, $A\ (=b+\beta)$, and $t_1\ (=c+\gamma)$ we obtain:

$$\varphi = a \log \frac{x}{b-x} + c - t + \frac{d\varphi}{d(1/K)}\alpha + \frac{d\varphi}{dA}\beta + \frac{d\varphi}{dt_1}\gamma = 0$$

Inserting the known values of a, b, and c, and the experimental values of x and t, we obtain a series of values of $a \log_{10} \frac{x}{b-x} + c - t$ which we may designate by the symbol $-\theta$.

$$\text{Hence: } \frac{d\varphi}{d(\frac{1}{K})}\alpha + \frac{d\varphi}{dA}\beta + \frac{d\varphi}{dt_1}\gamma = \theta \quad . \quad . \quad . \quad . \quad (1)$$

From the form of the equation:

$$\varphi = 1/K \log_{10} \frac{x}{A-x} + t_1 - t$$

[21] Cf. M. Merriman, A Text-book on the Method of Least Squares, 8th ed., New York, 1910, p. 200.

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it is evident that:

$$\frac{d\varphi}{d\,1/K} = \log_{10}\frac{x}{A-x}$$

to a very close approximation this may be written:

$$\frac{d\varphi}{d\,1/K} = \log_{10}\frac{x}{b-x} = \log_{10}\frac{x}{334-x}$$

Similarly:[22]

$$\frac{d\varphi}{dA} = \frac{-0.4343}{K\,(A-x)} = \frac{-6.5\times 0.4343}{344-x}$$

$$\frac{d\varphi}{dt_1} = 1$$

Computing the values of $\frac{d\varphi}{d\,1/K}$, $\frac{d\varphi}{dA}$, $\frac{d\varphi}{dt_1}$ and θ for each of the experimental values of x and t and inserting these values in equation (1) we obtain the following series of *obse vation equations*:

TABLE 8. MALES

AGE OF INFANT IN MONTHS	OBSERVATION—EQUATIONS	"WEIGHTS"
0	$-0.212\alpha - 0.0136\beta + \gamma = +0.003$	247
1	$-0.063\alpha - 0.0158\beta + \gamma = +0.035$	20
2	$+0.105\alpha - 0.0192\beta + \gamma = -0.058$	27
3	$+0.207\alpha - 0.0221\beta + \gamma = +0.279$	30
4	$+0.309\alpha - 0.0257\beta + \gamma = +0.616$	26
5	$+0.502\alpha - 0.0354\beta + \gamma = +0.362$	24
6	$+0.626\alpha - 0.0441\beta + \gamma = +0.556$	23
7	$+0.786\alpha - 0.0603\beta + \gamma = +0.516$	25
8	$+0.945\alpha - 0.0830\beta + \gamma = +0.482$	22
9	$+1.131\alpha - 0.1230\beta + \gamma = +0.273$	20
10	$+1.609\alpha - 0.3540\beta + \gamma = -1.834$	0
11	$+2.523\alpha - 2.8230\beta + \gamma = -6.775$	0
12	$+1.917\alpha - 0.7080\beta + \gamma = -1.836$	0

All of these observation-equations are not of equal value, since the first, that for birth, was derived from the determination of the mean weight of 247 infants, while the second, namely, that for one month, was derived from measurements made upon only 20 infants. Hence, in order to ascribe to each observation

[22] Since $\log_{10}\frac{x}{A-x} = 0.4343\,\mathrm{Log}_{\mathrm{nat}}\frac{x}{A-x}$

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its just value the equations must be *weighted* in proportion to the number of infants observed. This we do by multiplying every term in each equation by the number of infants observed.[23] I have, however, weighted the observations for 10, 11 and 12 months *zero*. This is for three reasons, namely: *First*, the small number of observations at each of these ages, were this the only ground for suspecting these observations it would be adequately provided for by weighting in proportion to that number, but there are also the following grounds for rejecting the data in estimating the true form of the curve of growth for the infantile growth-cycle. *Secondly*, at this part of the curve of growth the *second* extrauterine growth cycle (that which reaches its maximum velocity at 5.5 years[24] may be beginning to contribute an appreciable proportion to the growth of the infants and thus modify the form of the first growth cycle. *Thirdly*, small errors in the determination of x at this point lead to large errors in the estimation of $\frac{d\varphi}{dA}$ and therefore to large errors in the multipliers of β in the observation-equations which would result, if these equations were "weighted" in the same manner as the others, in giving slight errors in the determinations of mean weight at 10 to 12 months an undue effect in determining the most probable values of α, β and γ.

Proceeding in this way we obtain the following weighted observation-equations (Table 9).

TABLE 9. MALES.

AGE OF INFANT IN MONTHS	WEIGHTED OBSERVATION-EQUATIONS X Y Z θ
0	$-52.364\alpha - 3.360\beta + 247\gamma = + 0.741$
1	$- 1.260\alpha - 0.316\beta + 20\gamma = + 0.700$
2	$+ 2.835\alpha - 0.520\beta + 27\gamma = - 1.566$
3	$+ 6.210\alpha - 0.660\beta + 30\gamma = + 8.370$
4	$+ 8.034\alpha - 0.670\beta + 26\gamma = + 16.016$
5	$+12.048\alpha - 0.848\beta + 24\gamma = + 8.688$
6	$+14.398\alpha - 1.180\beta + 23\gamma = + 12.788$
7	$+19.650\alpha - 1.505\beta + 25\gamma = + 12.900$
8	$+20.790\alpha - 1.830\beta + 22\gamma = + 10.604$
9	$+22.620\alpha - 2.460\beta + 20\gamma = + 5.460$

[23] Cf. M. Merriman: loc. cit., p. 36.

[24] Cf. T. Brailsford Robertson: Arch. f. Entwicklungsmech., 25 (1908), p. 581.

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Calling the multipliers of α, β and γ x, y and z respectively, the "Normal-Equations" derivable from the above observation-equations are:

$$\alpha \Sigma x^2 + \beta \Sigma xy + \gamma \Sigma xz = \Sigma x\,\theta \ldots\ldots\ldots\ldots\ldots \text{I}$$
$$\alpha \Sigma xy + \beta \Sigma y^2 + \gamma \Sigma zy = \Sigma y\,\theta \ldots\ldots\ldots\ldots\ldots \text{II}$$
$$\alpha \Sigma xz + \beta \Sigma yz + \gamma \Sigma z^2 = \Sigma z\,\theta \ldots\ldots\ldots\ldots\ldots \text{III}$$

Computing the corresponding numerical values we obtain the equations:

$$4541.00\alpha + 16.82\beta - 10470\gamma = +1023.0 \ldots\ldots\ldots \text{I}$$
$$16.82\alpha + 25.92\beta - 1058\gamma = -91.5 \ldots\ldots\ldots \text{II}$$
$$10470.00\alpha - 1058.00\beta + 66330\gamma = +1990.0 \ldots\ldots\ldots \text{III}$$

Solving, we obtain:

$$\alpha = +0.853$$
$$\beta = +7.47$$
$$\gamma = +0.284$$

Hence the most probable values of:

$$1/K = a + \alpha = 6.5 + 0.85 = 7.35$$
$$A = b + \beta = 334 + 7.47 = 341.5$$
$$t_1 = c + \gamma = 1.375 + 0.284 = 1.66$$

Hence the equation to the curve of growth for the first nine months of the extra-uterine life of South Australian males is found to be:

$$\log_{10} \frac{x}{341.5 - x} = 0.136\,(t - 1.66) \ldots\ldots\ldots\ldots (2)$$

t being reckoned from the time of birth and x in ounces.

In the following table (Table 10) the observed weights at the various ages are compared with those calculated from the above formula.

The agreement between the observed and calculated values is obviously excellent. From the values of the deviations enumerated in the fourth column we can calculate the probable deviation of any observed value from its calculated value, employing the formula

$$\text{"Probable deviation"} = 0.6745 \sqrt{\frac{\Sigma p \Delta^2}{\text{Total number of observations}}}$$

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TABLE 10. MALES

AGE OF INFANT IN MONTHS	WEIGHT IN OUNCES		Δ=DEVIATION FROM CALCULATED VALUE
	Observed	Calculated	
0	127	127	±0
1	155	156	−1
2	187	180	+7
3	206	206	±0
4	224	230	−6
5	254	254	±0
6	270	273	−3
7	287	288	−1
8	300	301	−1
9	311	311	±0
10	326	319	+7
11	333	325	+8
12	330	330	±0

where p is the "weight" of a single observation and Δ the observed deviation.[25] In this way we find that the "probable deviation" of an observed from a calculated value, as indicated by the comparison in Table 10, is ± 1.5, which means that the agreement between theory and observation is such that as many observed values will be found to depart from the calculated value by *less* than 1.5 ounces as will be found to depart from the calculated value by *more* than 1.5 ounces. The corresponding computations for female infants follow (Tables 11 and 12), employing the approximate equation $8.0 \log \frac{x}{336 - x} + 2 = t$

TABLE 11. FEMALES

AGE OF INFANT IN MONTHS	OBSERVATION-EQUATION	"WEIGHTS"
0	$-0.250\alpha - 0.0162\beta + \gamma = \pm 0.000$	264
1	$-0.077\alpha - 0.0190\beta + \gamma = -0.384$	12
2	$\pm 0.000\alpha - 0.0207\beta + \gamma = \pm 0.000$	26
3	$+0.104\alpha - 0.0235\beta + \gamma = +0.168$	23
4	$+0.216\alpha - 0.0274\beta + \gamma = +0.272$	20
5	$+0.301\alpha - 0.0310\beta + \gamma = +0.592$	21
6	$+0.484\alpha - 0.0419\beta + \gamma = +0.128$	17
7	$+0.556\alpha - 0.0476\beta + \gamma = +0.552$	15
8	$+0.612\alpha - 0.0526\beta + \gamma = +1.104$	9
9	$+0.922\alpha - 0.0965\beta + \gamma = -0.376$	8

[25] Cf. M. Merriman, loc. cit., p. 82.

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TABLE 12. FEMALES

AGE OF INFANT IN MONTHS	WEIGHTED OBSERVATION-EQUATIONS X Y Z θ
0	$-66.00\alpha - 4.277\beta + 264\gamma = \pm 0.00$
1	$- 0.94\alpha - 0.228\beta + 12\gamma = - 4.51$
2	$\pm 0.00\alpha - 0.538\beta + 26\gamma = \pm 0.00$
3	$+ 2.39\alpha - 0.541\beta + 23\gamma = + 3.86$
4	$+ 4.32\alpha - 0.548\beta + 20\gamma = + 5.44$
5	$+ 6.32\alpha - 0.651\beta + 21\gamma = +12.43$
6	$+ 8.25\alpha - 0.712\beta + 17\gamma = + 2.04$
7	$+ 8.34\alpha - 0.714\beta + 15\gamma = + 8.28$
8	$+ 5.51\alpha - 0.473\beta + 9\gamma = + 9.94$
9	$+ 7.37\alpha - 0.772\beta + 8\gamma = - 2.94$

From these observation-equations we obtain the following normal-equations:

$$4643.0\alpha + 254.50\beta - 16790\gamma = + 234.50 \ldots\ldots\ldots \text{I}$$
$$254.5\alpha + 21.49\beta - 1216\gamma = - 21.93 \ldots\ldots\ldots \text{II}$$
$$16790.0\alpha - 1216.00\beta + 72545\gamma = + 629.30 \ldots\ldots\ldots \text{III}$$

Whence we obtain:

$$\alpha = + 0.99$$
$$\beta = + 13.75$$
$$\gamma = + 0.468$$

Hence the most probable values of:

$$1/K = 8.0 + 0.99 = 8.99$$
$$A = 336 + 14 = 350$$
$$t_1 = 2 + 0.47 = 2.47$$

Hence the equation to the curve of growth for the first nine months of the extra-uterine life of South Australian females is found to be:

$$\text{Log}_{10}\frac{x}{350 - x} = 0.111\,(t - 2.47) \ldots\ldots\ldots\ldots (3)$$

Comparing this equation with that (equation 2) found for males, we observe that although the infantile growth-cycle for females is no smaller in magnitude than that for males (i.e., the total growth due to it is no less when completed) yet it is slower in development, since K, which is a measure of the velocity of the

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growth-process, is only 0.111 for females, while it is 0.136 for males. Corresponding with this we find that the period at which the cycle is half completed is later (2.47 months after birth) in females than it is in males (1.66 months after birth). The fact that the period of gestation is slightly longer for females than for males is also doubtless to be attributed to the same factor.

In the following table (Table 13) the observed weights at various ages are compared with those calculated from the above formula.

TABLE 13. FEMALES

AGE OF INFANT IN MONTHS	WEIGHT IN OUNCES		Δ = DEVIATION FROM CALCULATED VALUE
	Observed	Calculated	
0	121	121	± 0
1	153	142	+11
2	168	164	+ 4
3	188	187	+ 1
4	209	209	± 0
5	224	230	− 6
6	253	249	+ 4
7	263	267	− 4
8	270	282	−12
9	300	295	+ 5
10	315	305	+10
11	335	314	+21
12	345	321	+24

The agreement between the observed and calculated values for the first nine months is very good. The "probable deviation" between observation and theory is ± 2.2 ounces.

7. The Relationship of the Post- to the Pre-Natal Curve of Growth for South Australian Infants

Having thus found an algebraical formula which adequately represents the post-natal growth of infants, as determined from direct measurements at varying periods succeeding birth, we can now proceed to exterpolate with the aid of this formula, that is to continue the observed curve a short distance backwards and ascertain whether and to what degree this exterpolated curve

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agrees with the curve of pre-natal growth determined by the measurement of the weights of pre- and post-maturely born infants at birth.

The mean period of gestation for South Australian male infants is, as we have seen, 282.5 days. In equation 2, which represents the post-natal growth of males, therefore (page 27), $t = 0$ when the infant has presumably lived 282.5 days of intra-uterine life. It is true that there is much doubt whether the period of gestation measured from the onset of the last menstruation represents the true period occupied by the growth of the embryo or not,[26] but this uncertainty does not attach in nearly the same degree to the estimation of *differences* between periods of gestation with which we are solely concerned here. Taking, therefore, the value of $t = 0$ for the period of 282.5 days, and recollecting that $t = 1$ at 30 days we find that male infants born, for example, at 260 days have been delivered 22.5 days or 0.750 month prior to the period defined by $t = 0$ equation 2. In other words in order to find by exterpolation from equation 2 the "calculated" weight of an infant of this age, that is the weight which it should have if intra-uterine growth is continued uninterruptedly into extra-uterine growth, we must insert the value $t = -0.750$ and calculate from the equation the corresponding value of x. Proceeding in this way we obtain a series of "calculated" weights which if intra- and extra-uterine growth are portions of the same growth-cycle, should agree very closely with the weights actually determined by weighing infants born at the corresponding periods. A comparison of the "calculated" and "observed" weights of male infants born at varying periods of gestation follows (Table 14), the "observed" weights being taken from Table 2.

The corresponding computations for females follow (Table 15) recollecting that in this case $t = 0$ at 284.5 days. The "observed" weights are taken from Table 4.

The agreement between the observed and calculated weights is as close as could possibly be expected. There is absolutely

[26] Cf. J. W. Williams: Obstetrics, 3d ed., New York, 1912, pp. 85, 86.

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no appreciable deviation of the prenatal curve of growth determined in the manner described from the continuation of the post-natal curve of growth backwards to cover the same period. This is also clearly revealed by a glance at the accompanying curves (figs. 1 and 2) in which the "calculated" curve of growth is represented by the curved line which is intersected at birth

TABLE 14. MALES

PERIOD OF GESTATION IN DAYS	t	WEIGHT IN OUNCES		Δ=DEVIATION FROM CALCULATED VALUE
		Observed	Calculated	
260	−0.75	111	110	+1
270	−0.42	117	117	±0
280	−0.08	127	126	+1
282.5	±0.00	127	127	±0
290	+0.25	137	134	+3
300	+0.58	145	142	+3
310	+0.92	146	151	−5
				$\Sigma\Delta = +3$

TABLE 15. FEMALES

PERIOD OF GESTATION IN DAYS	t	WEIGHT IN OUNCES		Δ=DEVIATION FROM CALCULATED VALUE
		Observed	Calculated	
250	−1.13	97	99	−2
260	−0.82	105	106	−1
270	−0.48	108	112	−4
280	−0.15	120	118	+2
284.5	±0.00	121	121	±0
290	+0.18	130	125	+5
300	+0.52	133	132	+1
310	+0.85	138	139	−1
				$\Sigma\Delta = +0$

by a heavy vertical line cutting the time coördinate at $t = 0$. The observed weights both for pre- and post-natal growth are indicated by small crosses (x). It is obvious that the "observed" curve exhibits no pronounced change in slope as it enters the period of intra-uterine development.

From these considerations and the curves which illustrate them it is evident: *Firstly* that the weights at birth of children

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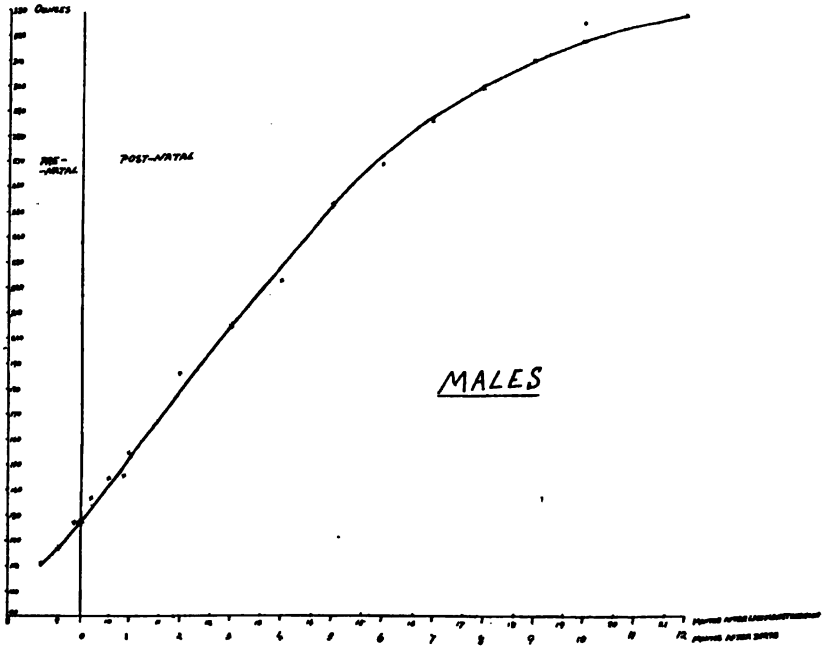


FIGURE 1

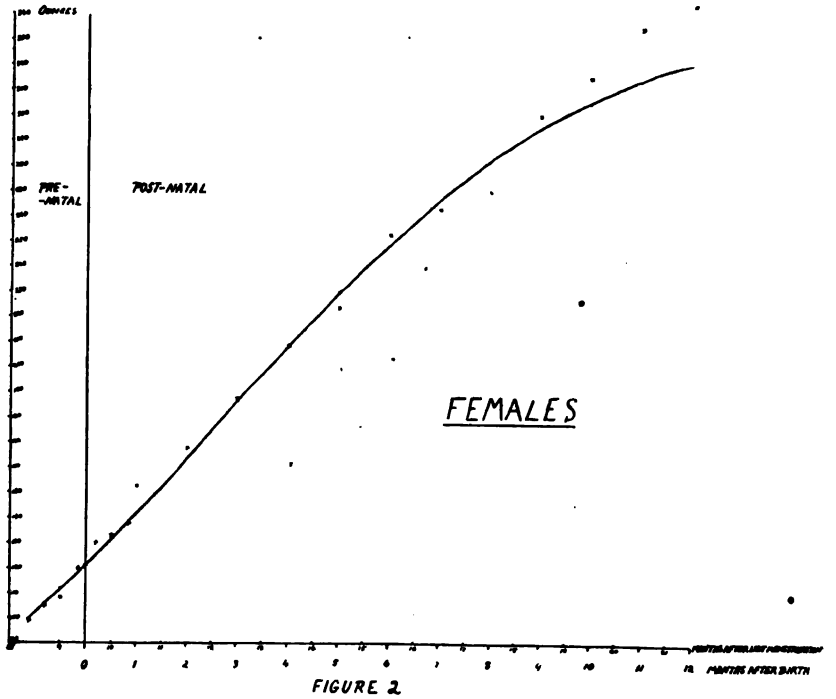


FIGURE 2

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which are born *after* the normal term are identical, within very narrow limits, with the weights which they would have attained at that time had they been born at the normal period, and *secondly* that the post-natal growth-cycle as determined from weighings of infants of from 1 to 9 months of age, is continued smoothly backwards into the pre-natal curve of growth. Hence there is no indication whatever of any other growth-cycle in the pre-natal growth of man, at least in the neighborhood of birth, and, from this and from the dimensions of the cycle, it appears very probable that the cycle of growth which is determining the rate of development at birth and during the greater part of the first year of extra-uterine life is merely a continuation of a cycle which begins at or very near to the moment of actual conception, very probably at the time of implantation of the embyo.

This cycle is accompanied by the production of tissues unusually rich in phospholipines[27] and is therefore of the type which I have elsewhere designated "autokinetic."[28] Preceding this cycle, and prior to the fixation of the embryo, we may assume that there is probably a very brief cycle of chiefly nuclear growth which, as the experiments of Robertson and Wasteneys indicate[29] must be accompanied by a *decrease* of phospholipines in the tissues and is therefore of the type which I have termed "autostatic." *Following* the cycle which is interrupted by birth is another cycle which attains its maximum velocity at about 5.5 years in both sexes[30] which, as Siwertzow has shown (loc. cit.) is accompanied by a diminution of the phospholipines in the tissues and is therefore of the "autostatic" type. Following this, again, and merging into it is the final cycle, of which the maximum velocity occurs at 14.5 years in females and 16.5 years in males and which culminates with the cessation of normal growth and the attainment of adult weight. Having regard to the alternation of auto-

[27] D. I. Siwertzow: Dissertation, St. Petersburg, 1904. Cited after Biochem. Zentralbl, Bd. 2. (1904), p. 310.

[28] T. Brailsford Robertson: Arch. f. Entwicklungsmech., 37 (1913), p. 497.

[29] T. Brailsford Robertson and Hardolph Wasteneys: Arch. f. Entwicklungsmechanik, 37 (1913) p. 485.

[30] T. Brailsford Robertson: Arch. f. Entwicklungsmechanik, 25 (1908), p. 581.

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kinetic and autostatic cycles which occurs during the first three cycles of growth it would appear probable, although no direct evidence exists, so far as I am aware, to this effect that this final growth-cycle is of the "autokinetic" type. Carcinomatous growth is demonstrably of the "autostatic" type[31] and it is therefore inviting to suppose that the growth of carcinoma represents an "autostatic" cycle superimposed upon the normally final "autokinetic" growth cycle of man. However this may be it is evident that there are probably four and certainly not less than three growth-cycles in the normal development of man, namely:

I. Very brief "autostatic," probably preceding implantation of the embryo.

II. Lasting from nearly the beginning of development until nearly the end of the first year of extra-uterine life. Maximum velocity at 1.66 months in males and 2.47 months in females, "autokinetic."

III. Starting during or close to the completion of the first year of extra-uterine growth and partially fusing into the succeeding cycle. Maximum rate at about 5.5 years in both sexes, "autostatic."

IV. Maximum velocity at about 14.5 years in females and 16.5 years in males. Culminating in the attainment of adult weight. Probably "autokinetic."

8. The Non-Existence of a "Critical Period" in the Normal Intra-uterine Development of Man

As I have stated in the introduction, Read (loc. cit.) has shown that there is certainly one, and there are possibly more growth-cycles which comprise the intra-uterine growth of the guinea-pig and culminate before the post-natal cycle begins. This latter cycle, in the guinea-pig is interrupted shortly after its commencement by birth. At the point of junction of these

[31] T. Brailsford Robertson and Theo. C. Burnett: Proc. Soc. Exper. Biol. and Medicine, New York and San Francisco, Dec., 1912, and April, 1913, Journ. of Exper. Medicine, 17 (1913) p. 244. T. Brailsford Robertson: Arch. f. Entwicklungsmech., 37 (1913), p. 497.

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cycles there is a "critical period" distinguished by a marked tendency to premature delivery of young, possibly through a failure of the two cycles to "link up" properly.

From the preceding considerations it is evident that there is no trace of such a juncture of cycles (at any rate subsequently to implantation) in the intra-uterine growth of man, and if the "critical period" in the intra-uterine growth of guinea-pigs is really attributable to the juncture of cycles with which it coincides, then since no such juncture occurs in human intra-uterine growth we might expect to find no critical period in the intra-uterine growth (subsequent to implantation) of the human being.

A tendency for premature deliveries to occur at a certain period rather than at any other would be presumptive evidence of a "critical period" in the intra-uterine growth of man. No such phenomenon occurs, however, notwithstanding the mistaken impression of some obstetricians to the contrary.[32] This is shown by the pronounced *unimodality* of the frequency-curve[33] for the period of gestation which is displayed by the following figures (Tables 16 and 17) derived from Tables 1 and 3.

There is evidently only *one* period, the "normal" period at which the percentage of infants delivered by normal mothers attains a maximum. Subsequently to implantation of the embryo there is no evidence of a "critical period" in the intra-uterine growth of man.

[32] It may be contended that by excluding those infants which died within one week of birth I have excluded the very group of deliveries which might be expected to reveal bimodality of the frequency-curve of the period of gestation. The deliveries thus rejected were, however, relatively few in number and displayed no special tendency to occur at a period different from the "normal" period of gestation. Their rejection is rendered necessary by the fact that they represent not infrequently the fruit of pregnancies which are affected by maternal abnormality. Were there any decided tendency, however, for deliveries, within the limits comprised in the accompanying tables (16 and 17), to fall into two groups, a certain proportion of the infants delivered at the abnormal period would certainly survive, since premature delivery within these or even more extreme limits is not an insuperable obstacle to subsequent development, and maldevelopment at a "critical period" of intra-uterine growth might be expected to occur in varying degrees resulting in the delivery of many infants not sufficiently maldeveloped to render the maintenance of life impossible.

[33] Cf. C. B. Davenport: loc. cit.

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TABLE 16. MALES

PERIOD OF GESTATION IN DAYS	PERCENTAGE OF ALL INFANTS NOT EXCLUDED BY CHAUVENET'S CRITERION (247) BORN AT THE DESIGNATED PERIOD
250	0.8
260	8.9
270	15.4
280	32.0
	— mode
290	31.6
300	6.5
310	3.6
320	1.2
	100.00

TABLE 17. FEMALES

PERIOD OF GESTATION IN DAYS	PERCENTAGE OF ALL INFANTS NOT EXCLUDED BY CHAUVENET'S CRITERION (264) BORN AT THE DESIGNATED PERIOD
240	1.1
250	2.3
260	3.8
270	12.1
280	30.3
	— mode
290	32.6
300	11.4
310	5.3
320	1.1
	100.0

9. The Existence of a "Critical Period" in the Latter Half of the First Year of the Extra-uterine Development of Man

Subsequently to the implantation of the embryo, the guinea-pig passes through a complete growth-cycle in utero and enters upon a second before birth. Man, on the contrary, is born before his first growth-cycle, subsequent to implantation, is half completed. Corresponding to this we find that the guinea-pig is born in a relatively adult condition of development.[34] It can run about and is not dependent upon its mother for nutrition within 4 days after birth. The "critical period" which some-

[34] J. Marion Read: Univ. of Calif. Publ. Zoology, 9 (1912), p. 341.

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what antedates birth in the guinea-pig, therefore, corresponds to a developmental stage which is not attained by a child until some time after birth. If the "critical period" in the intra-uterine growth of the guinea-pig originates in the difficulty with which a linkage of growth-cycles is accomplished, we should expect to find a similar "critical period" in the latter half of the first year of the extra-uterine growth of man, at which period, as a glance at figures 1 and 2 reveals, a notable "slackening off" of the growth-process occurs, preceding a fresh acceleration in the second year which represents a portion of a second extra-uterine growth-cycle.

That the latter half of the first year of extra-uterine growth is really a "critical period" in the development of man is shown by the investigations of Macgregor[35] who has determined the relationship of weight to age in over 1700 infants admitted to the City of Glasgow Fever Hospital during the years 1907–1908. Macgregor finds that for children under one year of age the weight on admission increases fairly uniformly from 3 to 6 months and is tolerably close to the average for Glasgow children of the same age. After 6 months, however, the curve of growth has its continuity suddenly broken, descends to a relatively low level and only regains its natural position as the end of the first year is reached. Evidently children who fall victims to zymotic disease during the second half of the first year of extra-uterine growth tend to be of markedly subnormal weight before any loss of weight due to the disease itself has occurred. Moreover the incidence of measles, whooping cough, and scarlet fever reaches a maximum at the eighth and ninth months while diphtheria and cerebro-spinal meningitis, although these diseases rarely occur during the first year show a maximum incidence during the sixth and seventh months of the first year. From these facts it appears *firstly* that there is a tendency for a certain proportion of infants to be of markedly subnormal weight between the seventh and the tenth months and *secondly* that these subnormally developed infants are selectively affected by certain

[35] A. S. M. Macgregor: Proceedings of the Royal Philosophical Society of Glasgow, 21st April, 1909.

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zymotic diseases. On comparing Macgregor's growth-curve of infants under one year of age who have contracted zymotic disease with Read's growth-curve of prematurely delivered guinea-pigs it is impossible not to be struck by their remarkable similarity.

It might be imagined that the tendency of a proportion of infants to exhibit underweight at from seven to ten months is attributable solely to the fact that weaning "normally" (i.e., physiologically) should take place at this period, that the digestive disturbances consequent upon change of diet affect a certain proportion of children more severely than others and that these children are selectively affected by zymotic disease. These considerations, however, do not suffice by themselves to explain the phenomena observed by Macgregor, for in the first place, as he points out, bottle-fed infants supplied with milk from the city milk-depots of Glasgow show no loss of weight during the seventh to tenth months at all comparable with the subnormality exhibited by the infants admitted to the fever hospital. Therefore bottle feeding does not affect average infants more adversely during the seventh to tenth months than at any other period. Now under the conditions pertaining in a modern city the weaning of infants is by no means usually delayed until the "physiological" period. From the data collected in Birmingham by H. W. Pooler[36] it appears that not over two-thirds of the children of poor parents are breast-fed, while a still smaller proportion of the children of wealthier parents are breast-fed. Among those infants which are breast-fed weaning occurs at a very great variety of ages. Infants of subnormal weight, owing to digestive disturbances, should therefore be distributed fairly evenly among all ages during the first year unless bottle feeding tends to have a more deleterious effect upon infants between seven and ten months of age than at any other period in the first year which, as we have seen, is not the case.

It may be pointed out, also, that it is undoubtedly by no means accidental that the "physiological age" for weaning approximately coincides with the termination of one growth-cycle and

[36] H. W. Pooler: Sixth Annual Report of the Birmingham Infants' Health Society, 1913, p. 33.

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the inauguration of a second. It is indeed highly probable that a change from one growth-cycle to a succeeding cycle involves profound changes in the metabolism of the organism and pronounced changes in physiological habit or mode of development might be expected to occur at periods coinciding with the deeper-seated metabolic changes. From this point of view it is noteworthy that whereas the physiological age of weaning is delayed in infants until the latter half of the first year of extra-uterine growth, a period after birth approximately coinciding in length with the period of gestation, in the guinea-pig, in which the period of gestation is 67 days, there is no definite "physiological" period of lactation, and weaning may take place within 4 days after birth without resulting in injury to the young.[37] We may infer that the need for a maternal supply of nutrition approximately coincides in duration, both in the guinea-pig and in man, with the "autokinetic" growth-cycle which succeeds the implantation of the embryo.

10. Summary

1. I have estimated the latter part of the curve of pre-natal growth and the earlier part of the curve of post-natal growth of South Australian infants from the weights of infants born somewhat prior to or later than the normal period of gestation.

2. The curve of early post-natal growth thus determined is identical with the post-natal curve of growth for South Australian infants determined by weighing the infants from birth to 9 months of age. The curve of pre-natal growth estimated in the above manner is identical with the continuation backwards of the curve of post-natal growth.

3. The mean period of gestation for 247 South Australian males is 282.5 days after the onset of the last menstruation with a "probable error" of ± 0.55 days; that of 264 South Australian females is 284.5 ± 0.57 days. The "standard deviation" of periods of gestation culminating in the delivery of males is 12.7 days, that of periods of gestation culminating in the delivery of females is 13.8 days.

[37] J. Marion Read: loc. cit.

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4. The mean weight of South Australian male infants at delivery is 127.3 ounces (= 3608 grammes) with a variability of 14.3 per cent; that of South Australian female infants at delivery is 121.2 ounces (= 3435 grammes) with a variability of 14.5 per cent.

5. British infants born in South Australia are from 8 to 10 ounces heavier at birth than British infants born in the British Isles. Anglo-American infants born in the eastern United States are intermediate, in weight at birth, between British infants born in the British Isles and those born in South Australia.

6. This superiority in weight of South Australian infants is maintained in varying proportion throughout the first year of post-natal growth.

7. There is no indication whatever of more than one "growth-cycle" (sigmoid curve of growth) during the intra-uterine growth of man subsequently to implantation of the embryo. This "growth-cycle" is interrupted by birth when it is not yet half completed and culminates towards the end of the first year of post-natal life. The magnitude of this growth-cycle (that is the extent of growth due to it) is approximately equal in males and females, but it is slower in development in females than in males.

8. There are probably four and certainly not less than three growth-cycles in the normal development of man, namely:

I. Very brief, "autostatic," probably preceding implantation of the embryo.

II. Lasting from nearly the beginning of development until nearly the end of the first year of extra-uterine life. Maximum velocity at 1.66 months in males and 2.47 months in females, "autokinetic."

III. Starting during or close to the completion of the first year of extra-uterine growth and partially fusing with the succeeding cycle. Maximum rate at about 5.5 years in both sexes, "autostatic."

IV. Maximum velocity at about 12.5 years in females and 14.5 years in males, culminating in the attainment of adult weight. Probably "autokinetic."

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9. There is no "critical period" in the intra-uterine development of man at which maldevelopment and premature delivery are more liable to occur, in infants born by normal mothers, than at any other.

10. There is a "critical period" in the latter half of the first year of the extra-uterine growth of man during which an exceptional proportion of infants are liable to be subnormal in weight independently of the time of weaning. These subnormal infants are selectively attacked by certain zymotic diseases. This "critical period" is probably attributable to a certain difficulty with which the two growth-cycles which meet at this period "link up" with one another.

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VARIATIONS IN CORONARY PRESSURE AND THEIR BEARING ON THE RELAXATION RATE OF THE VENTRICLES

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From the Physiological Laboratory of the Yale Medical School

Of the numerous theories which ascribe a suction pump action to the heart during the diastolic phase, one has singularly persisted. This theory, first advocated by Ernst Brücke, is based on the assumption that the reinjection of the coronary system during diastole with blood at a high pressure causes an active dilatation of the ventricular chambers.

Earlier observations bearing directly on this topic have yielded conflicting results.[1]

Martin and Donaldson,[2] Henderson,[3] Von den Velden[4] and Lehndorff[5] have established beyond doubt the purely passive nature of diastole. In view of their investigations, the opinion that coronary tension can to any extent influence ventricular dilatation becomes untenable. Yet the fact that this view persists in modern text books, justifies the present experiments.

The following extracts will serve to illustrate the arguments upon which this theory is based. Howell[6] in a discussion of the suction action of the heart says:

> The heart in contracting exerts a force greater than that of the blood in the coronary vessels, and probably, therefore, these vessels are emptied and their cavities obliterated in part. At the beginning of diastole

[1] For a review of the literature up to 1904, the reader is referred to E. Ebstein, Ergebnisse der Physiologie, Dritter Jahrgang, II Abteilung, pp. 121, 194.

[2] Martin and Donaldson: Studies from the Biological Laboratory, Johns Hopkins University, 1887, iv, 37.

[3] Yandell Henderson: American Journal of Physiology, 1906, xvi, 325.

[4] Von den Velden: Zentralblatt für Physiologie, 1906, xx, 73.

[5] Lehndorff: Deutsches Archiv für Klinische Medizin, 1914, cvi, 75.

[6] W. H. Howell: A Text Book of Physiology, 5th Edition, 1913, p. 551.

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they are reinjected with blood under a pressure of perhaps 100 mm. of mercury, and this fact seems to offer a probable explanation for a partial dilatation of the ventricular cavity and a production of negative pressure in the brief interval before the opening of the auriculo-ventricular valves.

In Hirschfelder's well known work,[7] this statement occurs:

> The walls of the heart are sufficiently rigid and sufficiently provided with elastic fibers to resume their shape like a rubber ball, and on the other hand, *the pressure in the coronary arteries tends to hold them distended as though by a wire frame.* (Italics mine.)

Although the latter view differs somewhat from the first as to the mechanics involved, the end result assumed in both instances is the same: an active dilatation of the heart dependent on coronary tension.

Howell and Ely[8] have shown, in the isolated heart of the dog, that the duration of systole and diastole is practically uninfluenced by variations in arterial pressure. In the absence of variations in diastolic time, if coronary tension is a factor in the production of an active diastole, changes in arterial pressure should cause variations in the relaxation or filling rate of the ventricles. On the basis of the "wire cage" hypothesis the rate of ventricular relaxation should bear some proportion to the coronary pressure as the elasticity of the cage would increase proportionately to the tension in the coronary vessels. Likewise, if the sudden increase in the turgidity of the heart walls which accompanies the inception of diastole is capable of aiding in the dilatation of the ventricular cavities, a relaxation rate proportional to the coronary pressure would be expected. The higher the coronary tension, the more rapidly should this erectile process take place.

In the present paper are given the results of experiments in which the behavior of the ventricles under variations of coronary pressure was studied, with special reference to their relaxation rate.

[7] A. D. Hirschfelder: Diseases of the Heart and Aorta, 1910, p. 11.

[8] Howell and Ely: Studies from the Biological Laboratory, Johns Hopkins University, 1882, ii, 453.

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The method used is, with slight modifications, similar to the one described recently by Henderson and Prince.[9] The advantages of this method for this particular problem lie in the ease with which the temperature, venous pressure and intraventricular systolic resistance can be controlled. The latter never exceeds the maximal diastolic pressure. This abnormally low resistance during systole, however, cannot be considered as detrimental to the results.

The experiments were performed with the excised heart of the cat. The animals were killed by decapitation and the heart removed after ligation of the pulmonary artery. A cannula was inserted into the severed aorta and perfusion immediately begun. The perfusion fluid consisted of equal parts of defibrinated sheep's blood and Tyrode's solution, impregnated with a half volume of CO_2 and with oxygen to the point of saturation. Extreme care was taken to prevent variations in temperature, all fluids coming in contact with the heart being kept at 37°C. Arterial pressure variations were obtained by air pressure applied directly to the perfusion fluid and recorded with a mercury manometer connected close to the aortic cannula. The intra-ventricular volume changes were obtained by means of a cylindrical vessel fitted at its inferior extremity with a glass cannula of suitable length and bore (fig. 1). This cannula was inserted through a slit in the auricular appendage into the ventricle. The closure of the auriculo-ventricular valve about this tube prevented regurgitation into the auricles. The oscillations of the column of fluid in the cylinder

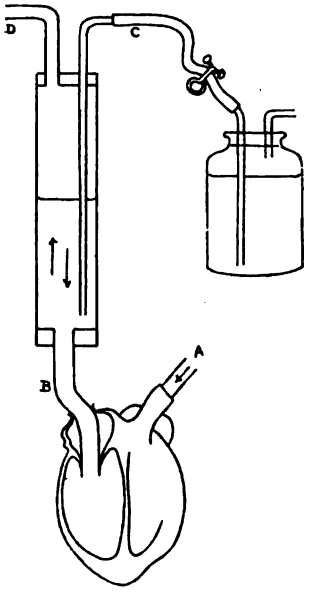


Fig. 1. A, Aortic cannula; B, Cannula inserted into ventricle; C, Syphon for the regulation of venous pressure; D, Tube to recording tambour.

[9] Henderson and Prince: Heart, 1914, v, 217.

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were recorded on a rapidly revolving smoked drum by means of a Marey tambour covered with loosely applied rubber dam and fitted with a very light lever. The venous pressure, as indicated by the mean height of the fluid in the cylinder was maintained at 150 mm. saline in experiments on the left ventricle and at 50 mm. on the right. These pressures, as shown by Henderson and Prince[10] are the optima for the isolated cat's heart. The following determinations were made: 1. The heart

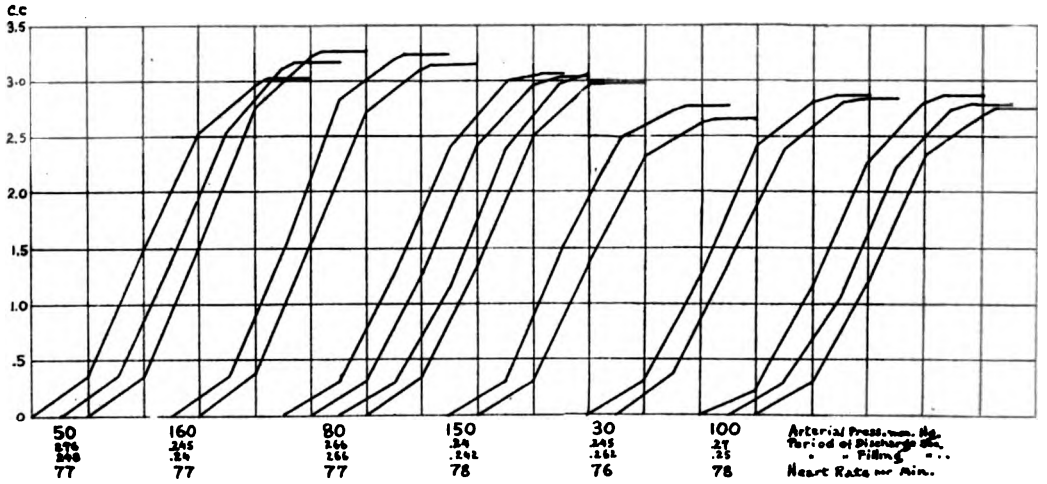


Fig. 2. Experiment I. Right ventricle. Venous pressure 50 mm. saline. Curves showing the relaxation rate of the right ventricle under variations of arterial pressure. Data obtained from intraventricular volume tracings. Under each group of curves are given the arterial pressure, the average duration of systolic discharge and diastolic filling and the heart rate. The intervals between adjacent ordinates represent 0.059 sec.

rate. 2. The duration of relaxation or filling of the ventricle (Diastole minus Diastasis). 3. The rate of relaxation of the ventricle (in cubic centimeters per equal intervals of time).

Observations were taken at intervals never more than three minutes apart, as in the excised heart the coronary flow gradually decreases in proportion to the length of the experiment. Controls on the rate of perfusion showed that this factor becomes negligible in view of the short duration of each series of observations, the total time never exceeding thirty minutes.

[10] Henderson and Prince: Heart, loc. cit.

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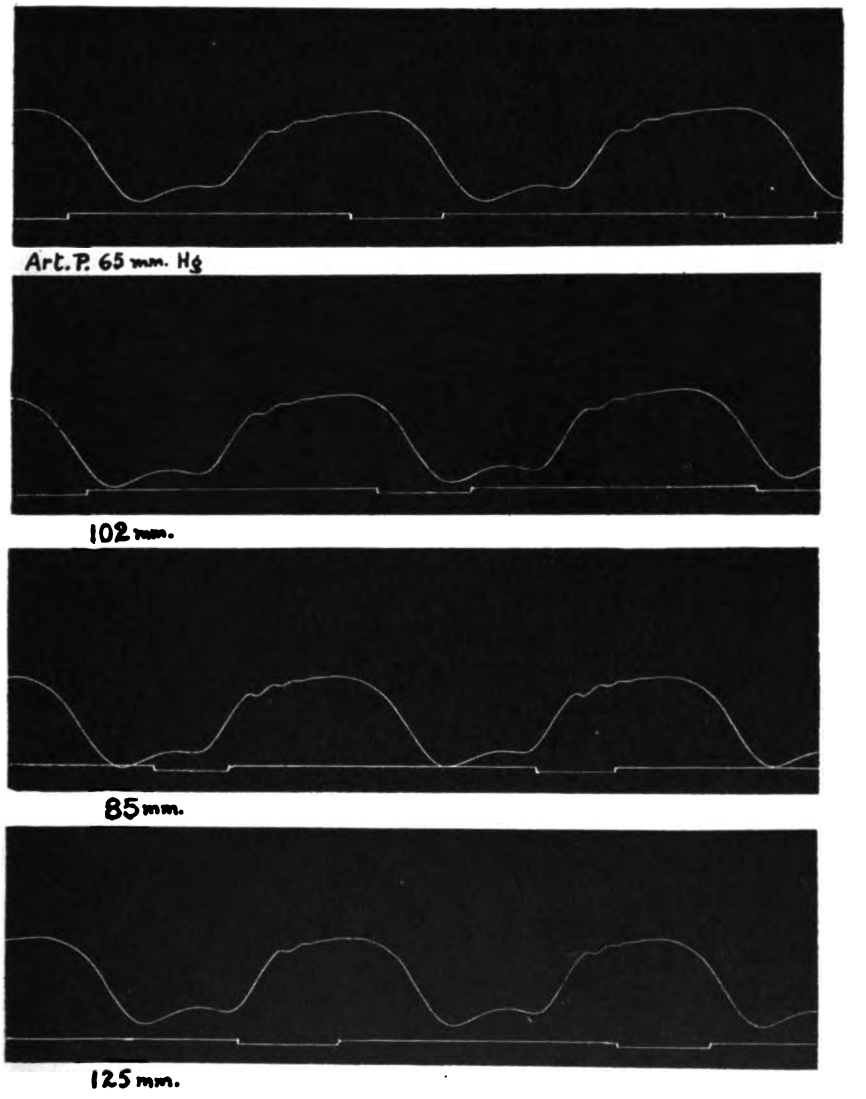


Fig. 3. Experiment II. Left ventricle. Venous pressure 150 mm. saline. Note uniformity of diastolic relaxation in the presence of marked variations in the duration of systole. Upstroke: Systole. Downstroke: Diastole.

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In Experiment I (fig. 2), on the right ventricle, the arterial pressures successively applied were: 50, 160, 80, 150, 30, and 100 mm. Hg. Slight variations in the rapidity of ventricular relaxation occur, which, however, bear no relation to the degree of coronary tension. As a whole the results are negative.

In Experiment II (fig. 3), on the left ventricle, the successive pressure changes were: 65, 102, 85 and 125 mm. Hg. As in all other observations the diastolic relaxation rate, although showing moderate variations, bears no relation to the coronary pressure changes.

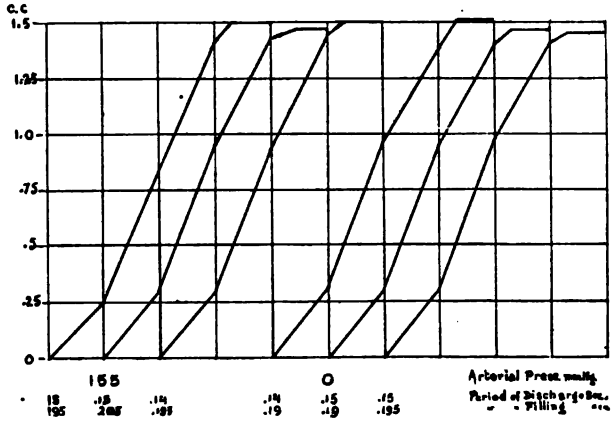


Fig. 4. Experiment III. Right ventricle. Venous pressure 50 mm. saline. Arterial pressure lowered from 155 to 0 mm. Hg. In this diagram the intervals between adjacent ordinates equal 0.059 sec.

Five other experiments on the right and left ventricles yielded similar results.

Experiment II is interesting from the standpoint of the time relations of systole and diastole. The heart exhibited a state of unusually high tonus with marked prolongation of systole. In this case the systolic time showed a distinct decrease at the higher pressures whereas the diastolic time remained practically unchanged. This observation speaks strongly in favor of a purely passive diastole, not associated with the active me-

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tabolic changes formerly ascribed to this phase of the cardiac cycle by certain investigators.[11]

This experiment is in marked discrepancy with the observations of Howell and Ely[12] but is so exceptional as not, I believe, to invalidate the generality of their conclusions.

It has been shown by Knowlton and Starling[13] and others, that at excessively low arterial pressures the efficiency of the heart is markedly impaired. If, however, the heart is subjected to these low pressures only for a short period of time, this period being preceded by a phase of normal pressure, maximal efficiency can be obtained for a few beats at an arterial pressure of zero.

To determine the possible effect of extreme coronary tensions on the rate of diastolic relaxation, the arterial pressure was suddenly reduced from 155 to 0 mm. Hg. in an experiment on the right ventricle (exp. III, fig. 4) and from 140 to 0 mm. Hg. on the left. The same negative results were obtained.

CONCLUSIONS

The relaxation rate of the ventricles is not affected by variations in arterial pressure. This speaks against theories ascribing to the heart a suction action brought about either by the tension or changes of tension in the coronary vessels.

The expenses of this research were defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

[11] Cited by E. Ebstein: loc. cit.
[12] Howell and Ely: loc. cit.
[13] Knowlton and Starling: Journal of Physiology, 1912, xliv, 206.

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CONTRIBUTION TO THE PHYSIOLOGY OF THE STOMACH

XXI. The Secretion of Gastric Juice in Man

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Received for publication, February 18, 1915

The subject in this study, Mr. F. V., is now 29 years old, and weighs 69 kilos. He has been in good health since he entered the service of the University three years ago in the spring of 1912. His hunger and appetite and his food consumption are those of the average man of his age, body weight, and physical activities. Three years ago Mr. V's stomach was described by Dr. Potter as of the "orthotonic" type (9), and from continued observations on the tonus and the motor activities of his empty stomach, I feel certain that his stomach is in this condition today.

The reader will recall that Mr. V. has had a complete cicatricial stenosis of the oesophagus and gastrostomy since the age of seven (9). He masticates all his food in the normal way, places the masticated mass in a syringe, and in that way introduces it into the stomach through the large rubber tube kept permanently in the gastric fistula. The general oral sensibility and the gustatory and olfactory senses of Mr. V. show normal range and activity. In brief, Mr. V. is a normal man, except for the gastrostomy and the closed oesophagus. This fact is of importance, as it permits us to use Mr. V. as a type for the secretion and the chemistry of gastric juice in adult normal persons.

The present report deals only with the secretion of gastric juice. A later paper will include the studies on the chemistry of the hunger and the appetite secretions. Most of the present work was completed during June–October, 1912, but all the points

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were checked up and extended during June–August, 1914, and during January–February, 1915. I thus have a great number of observations made at different seasons of the year, covering a period of three years. This should eliminate all variations due to special conditions.

METHODS

Most of the observations were made in connection with the noon day meal, that is, from 11 a.m. to 4 p.m. Mr. V's customary breakfast is eaten at 7 a.m., and consists of 3 to 4 biscuits (250–300 gr.), and about 200 cc. milk in about the same quantity of coffee. This meal is practically all out of the stomach in 3 to 3½ hours. At 10 or 10.30 a.m. 150 cc. of lukewarm water is put into the stomach, to insure complete emptying, and an hour later the observations are begun on the hunger or the appetite secretion. The evening meal is eaten at 6.30 or 7 p.m. In case of all observations in connection with the evening meal, the lunch (meat, potatoes, bread, milk, and fruit or pastry) was eaten not later than 12.30 p.m. and 150 cc. of lukewarm water was put into the stomach at 5.30 p.m.

The collection of the gastric juice. The permanent tube in the fistula extends far into the stomach cavity, and is provided with a number of slits so as to permit free access of the gastric juice. Slight pressure on the abdomen permits complete emptying of the stomach when Mr. V. is sitting or standing. The best results are obtained by draining the stomach at five or ten minute intervals, rather than letting the juice escape continuously through the open tube. This is particularly true of the observations on the hunger secretion which is ordinarily very scanty. This method has also the advantage of permitting Mr. V. to be busy with various tasks in the laboratory during the tests, as in that way his cerebral processes are more satisfactorily controlled. In many cases, however, the appetite secretion was measured by continuous outflow from the tube while Mr. V. was masticating his food, sitting down at the table, or standing up by an improvised lunch counter.

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I. THE FLUID CONTENTS OF THE STOMACH FREE FROM FOOD

The normal stomach, empty of food, always contains some fluid and mucus. The normal stomach is therefore, strictly speaking, never empty. This fluid in the empty stomach is made up of (1) gastric juice, (2) saliva, (3) duodenal contents (pancreatic juice and bile). Pancreatic juice and bile are frequently absent, however. The total fluid content of the empty stomach as well as the chemistry of this fluid depends on several factors, such as the relative rate of gastric and salivary secretion, the tonus and contractions of the stomach, the rate of absorption in the stomach, and the rate of emptying of the stomach contents into the duodenum.

According to the more recent literature the fluid content of the empty stomach of normal persons varies within wide limits. Verhaegen found the average to be 10–25 cc., but occasionally as much as 50 cc. was obtained. Moritz gives higher figures, or 24–64 cc. Working on himself Moritz obtained an average of 43 cc. of fluid in the stomach in the morning, with an acidity of 0.11 per cent. Moritz points out that it is not always possible to completely empty the stomach of fluid by the ordinary stomach tube. Rehfus, Bergheim and Hawk have very recently reported figures on this in normal persons, the fluid in the stomach at 8 a.m. in the morning varying from 30 cc. to 180 cc. It is not clear from their data whether these figures represent one test or the average of a great number of tests on each person. If they represent only one test on each person, they are of doubtful value, as there might be considerable dilation of the stomach with influx of bile, if the person is not used to swallowing the stomach tube; and unless the persons were used to going without breakfast there would in all probability be considerable secretion of appetite gastric juice in the morning by the persons merely thinking of food. It is obvious that the continued finding of 150–180 cc. of fluid in the empty stomach of healthy adults would seriously question the generally accepted view of clinicians that in health the fluid in the empty stomach (in the morning) should not greatly exceed 20 cc.

My own results on Mr. V. are presented in Table I. There

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are in all the groups a sufficient number of tests to give value to the average figures. It might be stated that most of these tests were made in connection with other lines of inquiry (hunger mechanism, action of bitters, appetite secretion, etc.), and not primarily for the purpose of determining the fluid contents of the empty stomach.

TABLE I

Fluid contents of the empty stomach of Mr. V. at different times of the year and the day

DATE	TIME OF DAY	NO. OF TESTS	CONTENTS OF EMPTY STOMACH, CC.		
			Low	High	Average
1912 June–August.....	Noon	45	10	35	19
1914 June–October.....	Noon	81	8	40	18.6
	Evening	55	9	36	16.2
1915 Jan.–February.....	Noon	20	8	17	12
	Morning	25	13	38	23

The data in Table I seems to show that:

1. The fluid contents of the empty stomach of Mr. V. varies considerably from time to time, but that the general average does not exceed 20 to 25 cc.

2. The gastric content is on the whole greater during the summer than during the winter months.

3. The gastric content is more abundant in the morning before breakfast than at noon before lunch.

In the case of Mr. V. the swallowed saliva is not a factor, as the oesophagus is completely closed. We are therefore dealing only with the factors of gastric tonus, continued gastric secretion, and the entrance of duodenal content. I am inclined to think that the gastric tonus is the most important item. The continuous secretion of gastric juice in the empty stomach of Mr. V. varies in rate from 2 cc. to 50 cc. per hour, yet the fluid content of the empty stomach may be as great with the low as with the high rate of secretion. Apparently, it is a question not only of rate of gastric secretion but also of the rate of emptying into the duodenum, and this passage of the gastric contents into the intestines depends directly on the gastric tonus. In all probability it is this factor (gastric tonus) which is mainly responsible

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for the greater abundance of the gastric content in the morning than at noon or evening, and during the summer in comparison with the winter months. So far as I know, however, we have no direct proof that the tonus of the empty stomach is less in the early morning than later in the day, and less during the warm summer than during the cold winter months. But since vigorous activity leads indirectly to increased tonus of the empty stomach through some change in the blood (10), the above surmise is more than a guess. It is also, I think, a fairly uniform experience that after a night of restful sleep the hunger sensation, which depends directly on gastric tonus (11) is feebler than later in the day. It is the author's experience that, partaking of dinner at 6 p.m. the hunger sensation is stronger at 11–12 o'clock at night before going to sleep than at 5–7 in the morning after a night's rest, despite the fact that in the morning the stomach has been free from food for a longer time.

The contents of the empty stomach of Mr. V. always contains some free HCl and pepsin. But the acidity is low, never exceeding 0.2 per cent, and frequently as low as 0.05 per cent. In the morning the gastric content is frequently, during the day rarely, mixed with bile. This also points to a greater tonus relaxation of the stomach in the morning.

II. THE CONTINUOUS SECRETION OF THE EMPTY STOMACH

Continued secretion of gastric juice in the absence of food in the alimentary tract, and in the absence of cerebral processes relating to appetite ("psychic" stimulation), is a well known phenomenon in certain types of gastric disorders, but it is generally assumed by physiologists that in the absence of psychic stimulation, the gastric glands cease to secrete almost as soon as the stomach is emptied of chyme, and that the glands remain practically quiescent up to the next feeding. The quiescence is supposed to be sufficiently complete to render the surface of the stomach alkaline, due to the continued secretion of alkaline mucus. To the extent that this view is anything more than an assumption, it is based essentially on the studies of Pawlow and his pupils on dogs. Pawlow frequently emphasizes the

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fact that not a drop of gastric juice flows from the stomach unless there is food or other stimuli in the stomach or unless the appetite mechanism is called into play.

Later Boldyreff (4) reported that on continued starvation the gastric glands exhibit periodic activity, and if the starvation is maintained for more than three or four days the secretion of the gastric gland becomes continuous. In gastric fistula cases of normal persons no specific study has been made of the continuous secretory activity of the empty stomach, so far as I can learn from the literature, but in some instances (Kaznelson, Hornborg) there are indications of a slow continued secretion even when the stomach had been free from food for hours. In the first report (9) of the studies on Mr. V. in 1912 it was noted that "during the hunger contractions of the empty stomach the secretion of mucin is increased, and there is a decrease of hydrochloric acid, but the secretion is rich in pepsin. In no instance was the stomach found free from gastric juice, that is a fluid containing pepsin, free hydrochloric acid and mucin in varying concentrations. There are periods of spontaneous secretion of gastric juice in the empty stomach, the acidity of which is nearly equal to that of the psychic secretion." During the five days complete starvation experiment on Mr. L. and the author both of us noted that our stomachs contained some acid gastric juice night and day throughout the entire period, but as this observation was incidental we did not determine the rate and quantity of this continuous secretion (12).

Last year Rehfus, Bergheim and Hawk, working on apparently normal persons and using the Ewald meal and the Rehfus stomach tube, reported many cases in which the secretion of gastric juice continued for one-half to one hour or more after all the food had left the stomach. Unfortunately they give no data on the rate or quantity of this secretion, or the methods by which the appetite or psychic factor was controlled. It is not even clear from their report whether or not the stomach was completely emptied of this juice at each test. The acidity figures reported show that the percentage of hydrochloric acid of the spontaneous secretion was practically the same as that of the test breakfast chyme. The acidity is much too low for

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pure human gastric juice. Their gastric content was therefore mixed with saliva or pancreatic juice, or else the rate of the gastric secretion did not exceed 10–12 cc. per hour.

Most of my tests on Mr. V. were made between 10 am. and 4 p.m., the usual breakfast of coffee, milk and biscuits being taken at 7 a.m. A few tests were made 9–12 a.m., in which case Mr. V. did not take any breakfast. The following typical results may be cited as illustrations:

Experiment 25, showing maximum rate of secretion of empty stomach. Breakfast 7 a.m.; 100 cc. water into stomach at 10 a.m.

11.00 a.m.	12 cc. in stomach, clear
11.10	8 cc. in stomach, clear
11.20	7 cc. in stomach, clear
11.30	8 cc. in stomach, clear
11.40	7 cc. in stomach, clear
11.50	8 cc. in stomach, clear
12.00 m.	6 cc. in stomach, clear

Starting to masticate a palatable lunch.

12.10 p.m. = 38 cc. gastric juice + trace of bile.

Experiment 17, showing an average rate of secretion of empty stomach. No breakfast; water into stomach at 7 a.m. and 10 a.m.

11.00 a.m.	10.0 cc. in stomach, clear
11.10	2.0 cc. in stomach, clear
11.20	1.0 cc. in stomach, clear
11.30	0.5 cc. in stomach, clear
11.40	0.3 cc. in stomach, clear
11.50	0.5 cc. in stomach, clear
12.00 m.	1.0 cc. in stomach, clear
12.10 p.m.	1.0 cc. in stomach, clear
12.20	0.5 cc. in stomach, clear
12.30	0.3 cc. in stomach, clear
12.40	0.3 cc. in stomach, clear
12.50	0.4 cc. in stomach, clear
1.00	0.5 cc. in stomach, clear
1.10	1.0 cc. in stomach, clear
1.20	0.5 cc. in stomach, clear

Starting to eat lunch.

1.30 = 22 cc. gastric juice, clear.

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Experiment 45, showing minimum rate of secretion of empty stomach. Breakfast 7 a.m.; water into stomach at 10 a.m.

10.30 a.m.	12.0 cc. fluid in stomach
11.00	1.5 cc. gastric juice
11.30	1.5 cc. gastric juice
12.00 m.	1.0 cc. gastric juice
12.30 p.m.	1.5 cc. gastric juice

Starting to masticate the lunch.

12.40 = 46 cc. gastric juice

Experiment 4, showing spontaneous fluctuations in the rate of secretion of the empty stomach. Breakfast 7 a.m.; water into stomach 10 a.m.

12.00 m.	16 cc. fluid in stomach
12.30 p.m.	3 cc. gastric juice
1.00	4 cc. gastric juice
1.30	3 cc. gastric juice
2.00	8 cc. gastric juice
2.30	10 cc. gastric juice
3.00	7 cc. gastric juice
3.30	4 cc. gastric juice
4.00	1 cc. gastric juice

Starting to masticate lunch.

4.10 = 35 cc. gastric juice.

In general more gastric juice is obtained from the empty stomach, if the stomach is emptied every 5 or 10 minutes, than if it is emptied every 30 or 60 minutes. It is therefore likely that some of this secretion passes into the intestines or is actually reabsorbed in the stomach itself. It does not seem probable that the presence of a certain amount of this juice in the stomach would tend to inhibit further secretion.

The chemistry of this continued secretion will be reported on in a later paper in connection with that of the appetite gastric juice. If the secretion rate is low the free acidity is usually not over 0.20–0.25 per cent, but the pepsin concentration is nearly as great as that of the appetite gastric juice. If the secretion rate is 2–4 cc. in 10 minutes, the acidity is greater and the pepsin concentration may even exceed that of the appetite secretion. When the secretion rate is low the juice is very thick and opales-

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cent, owing to the great amount of ropy mucin. The viscosity is so great that it is difficult to handle small quantities of this juice in test tubes or pipettes.

What constitutes the stimulus to the continuous gastric secretion?

1. I think it can be shown that it is not an appetite secretion. To be sure, in the case of normal and vigorous persons periods of hunger contractions and appetite sensation are present almost as soon as the stomach is emptied of food. And it is obviously difficult to so control the cerebral processes of a person that the thoughts are not diverted to food and eating, especially if it is passed the usual meal time and one's attention is at times on the stomach. This is especially true if the gastric juice is collected every 10 minutes. If the stomach is emptied every 30 or 60 minutes and the person kept very busy with matters not pertaining to food and eating I think this factor is entirely eliminated. This was done every day for two weeks at a stretch, so as to make it a mere incident or routine in the day's work. Nevertheless, the continued secretion persisted with the usual fluctuations in character and quantity. I therefore feel that the conscious appetite or psychic factor is eliminated in most of these experiments.

2. Is the secretion due to a sub-conscious vagus tonus? The vagi carry secretory fibers to the gastric glands. But we know next to nothing about the reflex or tonus control of this neuro-secretory mechanism. We know that the vagi send tonus impulses to the gastric motor mechanism (8, 13). But it does not follow that this is also the situation in regard to the gastric glands. The mechanism governing the vagus tonus has interested the writer for some time. The possible secretory vagus tonus must be subjected to direct experiments.

3. The presence of food in the intestine may be partly responsible for this continued secretion, by reflex action from the intestinal mucosa (Pawlow), or by absorptions of gastric secretions into the blood. In a 59 year old man with gastric fistula Umber obtained some secretion of gastric juice on rectal feeding with milk, sugar and eggs. Umber explains the secretion as a reflex ef-

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fect from the mucosa of the large intestine. I am not convinced that purely psychic factors are excluded in his experiments. If a person is hungry it is likely he will be lead to think of food and eating by the mere act of rectal feeding. Moreover, Umber's experiments were not munerous enough to really establish the point.

4. Gastric juice itself contains mucins and proteins that are digested by the pepsin-hydrochloric of the gastric juice. It is highly probable that the acid products of this digestion yield gastric secretagogues, just as in the case of some of the digestion products of the food proteins. According to Bickel (2) amino acids given by mouth cause secretion of gastric juice. The recent work of Folin and Lyman appears to show definitely that nitrogenous digestion products are absorbed in the stomach itself. Absorbed slowly in the stomach or passed into the intestines to be absorbed there, the products of the autodigestion of the gastric juice probably furnishes chemical stimuli for a slow, but continuous gastric secretion. Which one of the above factors is of prime importance in the continuous secretion of gastric juice by the empty stomach must be determined by other lines of work.

III. THE APPETITE SECRETION OF GASTRIC JUICE

1. *The mere act of chewing indifferent substances, and the stimulation of nerve endings in the mouth by substances other than those directly related to food cause no secretion of gastric juice.*

On the above points my results on Mr. V. are in complete accord with those of Pawlow and his school on dogs, and contrary to those of a number of observers on man. The results on Mr. V. may be illustrated by the following typical experiments, presented in detail in Table II.

Richet reports secretion of gastric juice from acid stimulation in the mouth in a woman with gastric fistula and oesophageal stenosis. He also states that the introduction of food or sapid substances into the stomach via the fistula caused salivation. This must have been a purely psychic effect, unless the procedure

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caused nausea. The subject was evidently a hypersensitive woman. I have never observed any of these effects on Mr. V.

In 1896 Schüle introduced the method of obtaining pure appetite gastric juice in man by emptying the stomach by means of a stomach tube, then chewing food for 15 minutes, and again emptying the stomach with the tube. He claims that the mere act of chewing and the tasting of such sapid substances as oil of peppermint, slices of lemon, and mustard cause secretion of gastric juice even in the absence of appetite. It may be remarked, however, that in the absence of depressor factors normal persons

TABLE II

Gastric juice in cc.

	TIME IN MIN.	EXP. 19	EXP. 7	EXP. 11	EXP. 27
Nothing in mouth	10	5	1.0	0.4	1.0
	10	7	0.8	0.4	0.8
	10	6	0.5	0.5	1.0
Chewing paraffin	10	5	0.4	0.2	0.9
Nothing in mouth	10	4	0.4	0.3	1.0
Vinegar in mouth	10	6	0.4	0.2	1.0
Nothing in mouth	10	5	0.5	0.3	1.0
Mustard in mouth	10	6	0.5	0.4	0.8
Nothing in mouth	10	4	0.4	0.2	0.8
Quinine in mouth	10	3	0.3	0.5	0.9
Nothing in mouth	10	5	0.3	0.3	1.0
Chewing food	10	50	24.0	17.0	44.0

invariably experience some hunger and appetite as soon as and as long as the stomach is empty of food. Troller, using Schüle's method, also reports that slices, of lemon, mustard, etc., in the mouth, as well as the mere act of chewing, cause secretion of gastric juice. In the majority of his experiments the secretion thus obtained is very slight (only about one-quarter that obtained on chewing bread), and in some of the experiments recorded in detail the acidity of the juice is so low that it must have been mixed with swallowed saliva. It is probably very difficult for the average person to avoid swallowing some saliva with mustard or acidic acid in the mouth for 10–15 minutes. And so far as I can make out from the report, Troller did not adequately control

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the rate of secretion in the empty stomach when the persons had nothing in particular in the mouth. Riegel cites the case of a professional cook, in whom chewing of food (beef steak) or slices of lemon caused no secretion of gastric juice. This man showed chronic digestive disorders, however. But Riegel suggests that the absence of appetite secretion was due to a kind of permanent fatigue of the taste-secretory mechanism in consequence of his work as cook. Hornborg, working on a five year old boy with gastric fistula and nearly complete cicatricial stenosis of the oesophagus, concluded that chewing indifferent, bad tasting or strong tasting (lemon) substances did not induce secretion of gastric juice. Umber obtained no gastric secretion by chewing indifferent substances (a piece of rubber), but in one experiment alcohol in the mouth gave a slight secretion (3 cc.). It must be noted that Umber's subject was a man 59 years old, who might have been in the habit of taking alcoholic beverages with his meals.

Kaznelson, and Bickel, working on a 23-year-old girl with gastric fistula and complete cicatricial oesophageal stenosis, report that all sapid substances (quinine, asafœtida, etc.) in the mouth, even those that give rise to disgust, initiate or augment the gastric secretion. Kaznelson cites one experiment with quinine (control experiment with water) from which she concludes that bitter substances in the mouth augment the secretion of gastric juice; but her actual figures (Tables III, *a* and *b*, p. 37, 38) show, if anything, the reverse. The total secretion of gastric juice for 80 minutes with the water control (sham drinking) was 43.7 cc., while the quinine experiment yielded only 37.6 cc. for the corresponding time.

How are the above contradictory findings to be accounted for? In view of the consistently negative results of Pawlow and his students on dogs, and of Hornborg, and the writer on man, it is my belief that the investigators who report that mechanical chewing and stimulation of the nerve endings of general sensation in the mouth cause secretion of gastric juice have not eliminated the factors of appetite, swallowed saliva, and variations in the rate of the continuous secretion of the empty stomach. In man

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the appetite factor is not easily controlled, except by a long series of tests in which the experimental procedure becomes a mere routine to the subject. There appears to be no direct, or unconditional reflex pathway from the mouth to the gastric gland. Unless the stimuli in the mouth initiate or augment the central processes that constitute the sensation of appetite there is no innervation of the secretory nerve fibres to the stomach. It must also be remembered that lemon juice, acidic acid, and mustard are ingredients of many food preparations, and hence may stimulate appetite secretion.

2. *The relatively slight and inconstant secretion of gastric juice produced by seeing, smelling or thinking of food.*

Bringing a tray of palatable food into the room in sight of Mr. V. has never yet caused secretion of gastric juice, no matter what the degree of hunger and appetite. It is probable that under these conditions the primary and normal effects of seeing and smelling the food are inhibited by the consciousness of the experiment, or possibly his main interest was not the food but the expiration of the experiment so that he might partake of the food. To more closely approximate normal conditions, Mr. V. was sent out to the nearby cafeteria to select the lunch that he knew he would eat shortly after returning with it to the laboratory, the rate of his gastric secretion being measured for 10 minute periods before going for the food, during the selection of, and after returning to the laboratory with it. A few typical tests secured in this way are given in Table III.

In the majority of these tests the act of selecting the ingredients for the noon day meal caused a slight and temporary augmentation of the secretion rate of the empty stomach. On the whole this augmentation was greater the greater the rate of the continuous secretion. But on some days the augmentation was absent, although Mr. V. was to all appearances in normal condition, felt hunger, and the cafeteria displayed the usual variety of food stuffs.

Pawlow reports that there are great individual variation in dogs in the amount of gastric secretion induced by seeing and smelling food. This is in all likelihood true of man, and I sus-

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pect that Mr. V. belongs to the group of individuals in whom the taste of the food is the all important factor in the psychic secretion of gastric juice. I have not been able to appreciably augment the continuous secretion in Mr. V. by inducing the thought of food, for example, during a test while he is busy with other work, arresting his attention, casually, by discussing with him the taste and ingredients of his favorite dishes.

Schüle states that seeing or smelling food causes no secretion of gastric juice in normal persons. This is directly contradicted by Bulawinzew, according to the review of his paper in the *Biochemische Centralblatt* (the original paper is not accessible

TABLE III

Secretion of gastric juice on seeing, smelling, and thinking of food when hungry

	TIME IN MIN.	GASTRIC JUICE IN CC.					
		Exp. 3	Exp. 8	Exp. 12	Exp. 15	Exp. 30	Exp. 45
Selecting the lunch at the cafeteria.	10	5	0.5	0.3	0.4	0.6	0.4
	10	7	0.3	0.4	0.5	0.5	0.3
	10	6	0.5	0.3	0.4	0.4	0.4
	10	14	1.0	1.0	3.5	0.5	1.0
	10	10	1.0	0.6	2.0	0.4	1.0
	10	5	0.5	0.5		0.3	1.0
	10	6	0.3	0.4		0.4	0.7

to me). This investigator emptied the stomach by means of the stomach tube, let the subject see or smell food, and again emptied the stomach. The gastric juice thus obtained had such low acidity (0.2 per cent HCl) that it must either have been the continuous gastric secretion or the appetite gastric juice mixed with saliva. There is nothing in the review to indicate that he controlled the continuous gastric secretion. Hornborg obtained no secretion of gastric juice from the 5-year-old boy on seeing or smelling food, probably because the child always became angry when not permitted at once to eat the food shown him. Cade and Latarjet report secretion of gastric juice induced by talking to the subject about her favorite food. This subject (a young woman) is exceptional in that she virtually had an accessory

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stomach, but the mucosa of the isolated stomach portion was directly exposed so that the collection of the secretions was rather difficult. Kaznelson, and Bickel, working with a 23-year-old girl with gastric fistula and stenosis of the oesophagus, reached the remarkable conclusion that anything which stimulated the olfactory sense induced secretion of gastric juice in the resting stomach. Thus they claim that smelling ammonia, acidic acid, and aromatic oils cause secretion of gastric juice. This I am absolutely unable to confirm on Mr. V. It is possible that in this young woman every gustatory and olfactory stimulus when manipulated by the investigators led to thoughts of food through idea associations.

3. *The gastric secretion induced by tasting and chewing palatable food*

(1) *The secretion rate.* I have now records of 156 tests of the appetite secretion during the 20 minutes mastication of the noon day meal. The particular ingredients of this meal were of his own selection, and varied from day to day. It usually included soup and some kind of meat and gravy, and always milk, and a dessert. These data may be presented in the following summary:

Secretion of gastric juice during 20 minutes mastication of palatable food.

Lowest = 30 cc.
Highest = 156 cc.
Average = 70 cc.
Number of experiments = 156

This gives an average rate of secretion of 3.5 cc. of gastric juice per minute. The maximum rate of secretion obtained at any time was 54 cc. in 5 minutes, or 10.8 cc. per minute; the lowest was 7 cc. in 5 minutes, or 1.4 cc. per minute. The secretion rate is proportional to the palatability of the food. Thus the secretion rate is nearly always highest in the last 5-minute period, when Mr. V. masticates the dessert, and on the day when the highest rate of secretion was noted (156 cc. in 20 minutes) Mr. V. stated that the lunch was "unusually fine."

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A few typical experiments are given in detail in Table IV.

Is the above rate and quantity of appetite secretion of gastric juice typical for normal adults? So far as I can make out Mr. V. is in normal health, except for infrequent periods of headache and nervousness, the etiology of which is obscure.

TABLE IV

Rate of appetite secretion of gastric juice of Mr. V. Detail of typical experiments

EXPERIMENT NO.	RATE OF SECRETION OF GASTRIC JUICE IN CONSECUTIVE 5 MIN. PERIODS. CC.									
	Before starting chewing			During chewing				On cessation of chewing		
20	1	0.5	0.8	10	15	14	20	5	3	1.0
31				11	18	17	23	10	6	2.0
35	0.5	0.6	0.7	15	16	15	18	8	4	1.5
55	3.0	2.0	3.0	20	22	21	30	15	6	6.0
86	0.2	0.2	0.3	5	20	18	20	9	3	1.0
94	0.2	0.3	0.2	6	11	15	12	3	2	0.5
120	0.2	0.2	0.1	6	28	20	29	8	6	2.0
150	0.2	0.3	0.2	22	54	35	45	20	15	8.0

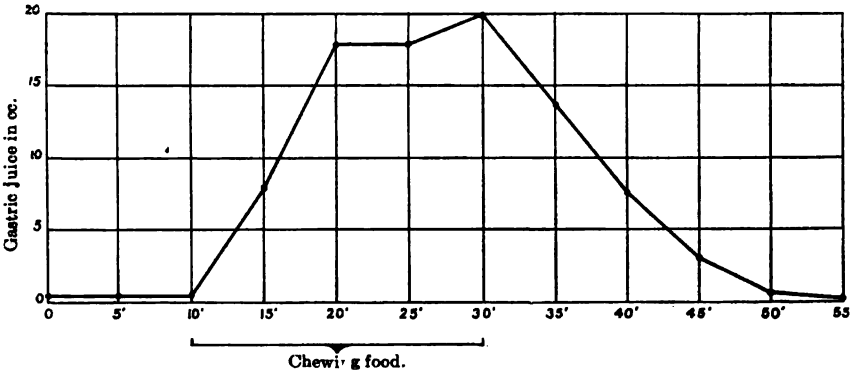


Fig. 1. Typical curve of secretion of gastric juice of Mr. V. on mastication of palatable food for twenty minutes. The gastric juice was collected at five minute intervals. The rise in the secretion rate during the last five minutes of mastication is due to chewing the dessert (fruit).

Troller reports five experiments on a person with nervous dyspepsia. Chewing beefsteak for 15 minutes yielded 55 cc. of gastric juice. Three experiments on a person with hyperacidity gave 50 cc. gastric juice in 15 minutes. This is a secretion of rate of about 3.5 cc. per minute. Chewing bread for 15 minutes yielded much less gastric juice. In the case of per-

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sons with hypochlorhydria, the average secretion for 15 minutes (chewing beefsteak) was only 28 cc. In Umber's fistula case (man 59 years old) two tests with chewing beefsteak for 15 minutes yielded 73 cc. and 48.5 cc. gastric juice in 60 minutes. This low rate of secretion (about 1 cc. per minute) must be due to the advanced age and to the malignant tumor of the oesophagus. The 10-year-old girl studied by Summerfeld secreted 110–150 cc. gastric juice in 90 minutes on chewing meat or mixed food for 30–40 minutes, a secretion rate of 2–2.5 cc. per minute. The maximum secretion rate in the 23-year-old girl studied by Kaznelson and Bickel, was 5 cc. per minute, the average secretion rate being much lower. Hornborg's 5-year-old boy secreted 15–25 cc. in 30 minutes on chewing meat or apple pie. Chewing bread or milk yielded less than half this amount. The 3-year-old child of Bogen on chewing meat for 15 minutes yielded 6–22.5 cc. gastric juice, or an average rate of less than 1 cc. per minute.

These data reported by previous investigators cannot be directly compared with my results on Mr. V. for the reason that the collection of the gastric juice was not always confined to the actual period of mastication of the food. The reader will observe on examination of Table IV that the rate of the appetite secretion starts to fall almost as soon as Mr. V. ceases to masticate the food and in 15 minutes the gastric glands are in most cases down to the level of the continuous secretion. The secretion rate is highest during the actual tasting of the food.

In this respect there is a marked difference between man and dog. In the dog after 12–24 hours starvation sham feeding with meat for 5 minutes may initiate and keep up secretion of gastric juice for 3–6 hours (Pawlow, Rosemann). It is obvious that in these tests on dogs the starvation period was much longer and the hunger and appetite more intense than in the present experiments on Mr. V. Another factor is probably the greater voluntary control over attention and other cerebral processes in man.

It may be of interest in this connection to note the rates of gastric secretion that have been obtained by sham feeding in dogs. Konowaloff reports 4 cc. per minute; Schoumow-Siman-

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owsky found a maximum of 5 cc. per minute; and Rosemann (in a 24 kilo dog) gives as the average 3.4 cc. per minute. Since the quantity of gastric glands even in very large dogs is probably only a third of that in the adult man, the above data seem to indicate that the gastric glands in dogs work with greater speed than the gastric glands of man.

The acidity and pepsin concentration of the gastric juice of Mr. V. are very constant. There has been practically no variations noted during the three years of observation. These will be reported on in a later paper.

(2) *The direct relation between the rate of appetite gastric secretion and the palatableness of the food.*

The mastication of bread and butter, or the taking of milk in the mouth yielded much less gastric juice than the chewing of meat. This is in line with results of previous observers on man. The taste nerve endings are evidently stimulated more intensely by the readily diffusible rapid substances in the meat. In general the desserts (pies, pudding, fruits) yielded even a greater secretion than meat. This was particularly noticeable in the case of chewing oranges. Mr. V. states that he is especially fond of oranges. The sapid substances in the orange juice probably diffuse readily and thus reach all the taste nerve endings in marked concentration.

There is no question but that the mastication of a palatable dessert at the end of a meal thus serves to augment and prolong the appetite secretion of gastric juice.

(3) *The latent period of the gastric appetite secretion.*

Pawlow and his co-workers found that the appetite gastric secretion in dogs exhibited uniformly a latent period of 5–6 minutes. The literature contains the following observations on the latent period in man.

Hornborg	3 minutes (meat)
Umber	3 minutes (meat)
Sick	6–10 minutes
Kaznelson	4–5 minutes
Bogen	4–5 minutes (meat) 9 minutes (milk)

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The work on Mr. V. has brought out the following facts:

1. The latent period of the appetite secretion depends primarily on the condition of the gastric glands. *Thus if there is a continuous gastric secretion of 2–6 cc. per 10 minutes at the time mastication of the food begins, the appetite secretion shows practically no latent period at all.* The quantity of gastric juice secreted during the first 5 minutes of chewing is just as great as that secreted during the second or third 5 minute periods. On the other hand, if the continuous secretion is very low (0.2–0.3 cc. or less per 10 minutes), the appetite secretion shows a latent period of 2–4 minutes. It is therefore evident that with the gastric secretion already in progress the appetite secretion reflex exhibits no greater latent time than the neuro-muscular reflexes in general.

2. The latent period varies indirectly with the intensity of the appetite stimulation. This fact is illustrated in Experiment 150, Table IV. In that case the continuous secretion was near the lowest mark, yet the first 5 minutes of chewing yields 22 cc. gastric juice, or more than the *average full secretion rate.* But even here a latent period of 2–2½ minutes is in evidence from the fact that the second 5 minutes period yielded 54 cc. gastric juice.

IV. THE TOTAL SECRETION OF GASTRIC JUICE IN MAN ON AN AVERAGE MEAL

As stated above Mr. V. yields appetite gastric juice at minimum secretion rate, 84 cc. per hour, maximum secretion rate, 648 cc. per hour, average secretion rate, 210 cc. per hour.

Does this furnish us a clue to the total gastric secretion on an average meal in man? This question cannot be answered by direct measurements, even in cases of duodenal fistula and collection of all the chyme issuing through the pyloric opening, as the alimentary tract of such persons is far from normal, and we still have the variable factors of swallowed saliva, and of direct absorption in the stomach. In the case of dogs sham feeding alone may yield 600–700 cc. of gastric juice in 4–6

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hours. But this situation is abnormal because the sham feeding does not satisfy the appetite, even though the secretion inhibits the hunger. It is therefore certain that the appetite secretion is much less when the food is permitted to reach the stomach. But when the food is allowed to reach the stomach how can we measure the total gastric secretion? Using large dogs with fistula of the duodenum, Moritz reports that the ingestion of 200 gr. of meat caused secretion of 320 cc. gastric juice in 7 hours. Part of this was undoubtedly swallowed saliva, and possibly some admixture of bile and pancreatic juice. With the same method Tobler obtained 200–300 cc. of gastric juice from feeding 100 gr. meat, part of which was undoubtedly swallowed saliva.

It seems to me that we can arrive at a very close estimate of the total average secretion of gastric juice in man on the following basis. Pawlow and his pupils have shown on dogs that the secretion curves of the main and the accessory stomach pouch run parallel. They have also shown that on a meal of meat or a mixed meal, the secretion usually reaches the maximum at the end of the first or during the second hour. Lönquist notes particularly that the secretion does not reach its maximum until toward the end of the second hour after feeding. This can mean only one thing, viz., that the rate of the hormone gastric secretion may be equal to or even exceed the rate of the appetite secretion.

On the whole, the quantity of gastric juice yielded by a dog's accessory stomach the first two hours on a moderate meal of meat, bread or a mixture of meat and bread, is about half of that secreted during the entire digestion period. This is evident from experiments reported in detail by Pawlow and his students, as well as from studies on dogs in our laboratory. This is not true if a very large quantity of food is given, or if the food contains a considerable amount of fat, as in both cases the secretion period is greatly prolonged.

I think we can safely assume that the general relations and the relative importance of the appetite and the hormane gastric juice are the same in man and dog. Pflaunder supports the view that the maximum rate of secretion in man is reached at the

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end of the first or the beginning of the second hour of digestion. Sick finds that the maximum acidity of the gastric content is reached at the end of the first hour of digestion. The same is shown by the more recent studies of Rehfus, Bergheim and Hawk using the Ewald test meal on normal persons. It is obvious, however, that the acidity curve of the gastric content after a meal does not give us a direct information on the question of gastric secretion rate, because of the variable factors of swallowed saliva, fixation of the HCl by the proteins of the food, rate of entrance of the chyme into the duodenum, and the entrance of duodenal contents into the stomach (Boldyreff). Nevertheless, so far as they go, these data on normal men are on the whole in agreement with the direct measurement on normal dogs with accessory stomachs.

The total secretion of gastric juice in normal adult man on ingestion of the average dinner of meat, bread, vegetables, coffee or milk and dessert will on the above assumption be as follows:

First hour	200 cc. gastric juice
Second hour	150 cc. gastric juice
Third to fifth hour	350 cc. gastric juice
Total	700 cc. gastric juice

It should be noted in this connection that Mr. V's noon day meal is in reality the big meal or dinner. I have evidence that he secretes less gastric juice on his evening meal, probably not more that 400–500 cc., (14) and from the fact that he makes his breakfast solely on biscuits, coffee and milk, it is likely that his secretion of gastric juice on the morning meal does not exceed 250–300 cc. This would make a total of 1350–1500 cc. gastric juice secreted in 24 hours. These figures do not include the continuous secretion in the absence of food. It is of interest to note that Pflaunder arrived at practically the same figures (1500 cc., or 25 cc. per kilo body weight, in 24 hours.), basing his estimate on calculations from the acidity and volume of the gastric content at varying periods after the meal.

It need not be pointed out that the above figures are sub-

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ject to great variations, depending on the food, the appetite secretion being determined primarily by the quality or taste of the food, while the hormone secretion is determined by the quantity and the chemistry of the food.

SUMMARY

1. The fluid contents of the "empty" stomach varies from 8 cc. to 50 cc. with an average of 20 cc. The quantity is greater in the morning than at noon or at 6 p.m. It is on the whole greater in the summer than in the winter months. The most important factor in these daily and seasonal variations is probably the tonicity of the empty stomach.

2. The gastric glands in the normal person are never completely quiescent. The continuous secretion varies from 2 to 50 cc. per hour. The higher figures are exceptional, but may obtain for several days in succession, again to revert to the lower figures. The vagus secretory tonus is a possible, and the autodigestion of the gastric juice itself is a probable factor in this continuous gastric secretion. The secretion itself is rich in pepsin, but when the secretion rate is very low it is poor in hydrochloric acid.

3. Chewing of indifferent substances, and stimulation of the nerve endings in the mouth by substances not related to food do not cause secretion of gastric juice, that is, these processes do not augment the continuous gastric secretion.

4. Seeing, smelling, and possibly thinking of palatable food usually cause a slight, but very transitory secretion of gastric juice.

5. The rate of secretion of gastric juice on mastication of palatable food is directly proportional to the palatability of the food. During mastication the average rate is 3.5 cc. per minute (minimum rate: 1.4 cc.; maximum rate: 10.8 cc.). On cessation of chewing the secretion rate diminishes rapidly so that in 15–20 minutes the gastric glands reach the level of the continuous gastric secretion. The chemistry of this appetite gastric juice has been practically constant during the three years of observation.

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6. The latent period of the appetite secretion varies indirectly with the rate of the continuous secretion, so that when the continuous secretion is abundant the appetite secretion shows no latent period at all, while with the lowest rate of the continuous secretion, the latent period varies from 2–4 minutes. This latent period is therefore one of the processes of secretion in the gland cells, and not in the nervous mechanism.

7. On the basis of these experiments on Mr. V. on the reports of other gastric fistula cases in man and on the work of Pawlow on dogs it is estimated that an adult normal person secretes on an average meal (dinner) 700 cc. gastric juice, or an average total of 1500 cc. gastric juice in 24 hours.

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STUDIES ON THE GROWTH OF MAN

2. The Post-Natal Loss of Weight in Infants and the Compensatory Over-Growth which Succeeds It

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1. INTRODUCTION

It is a familiar fact that for a variable period, in normal cases not exceeding one week, the majority of infants lose weight after birth. This is a phenomenon which is very generally observed after the birth of animals.[1] In the case of man it is usually attributed to the fact that for the first few days succeeding birth the child receives little or no nutriment.[2] That this is probably not the only factor involved, however, is shown by the fact that the post-natal loss of weight is usually much greater when the child is excessively large[3] and also by the fact that it occurs even in young guinea-pigs, which are born in such an adult condition that they are capable of feeding themselves entirely by the fourth day. It must be admitted, however, that the power of these animals to nourish themselves at such an early date assists them to overcome a large part of the post-natal retardation of growth, for the retardation is relatively slight in guinea-pigs and frequently leads to an actual loss of weight only in the male.[4]

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In the preceding article of this series,[5] I have presented a number of data concerning the weight of infants at birth after varying periods of gestation, derived from the records of "The Queen's Home," a maternity hospital in Adelaide, South Australia. In this institution the infants, whose mothers belong to the laboring and lower artisan classes, are weighed at birth and again upon discharge at from 13 to 15 days. Recently the practice has been instituted of also weighing the infants at one week after delivery, but only about one-third of the records which were placed at my disposal included the results of this weighing. My data concerning the weights of these infants at one week after birth are therefore less extensive than those which concern the weights at birth and at two weeks. They are, however, sufficiently numerous to permit the formation of certain conclusions which throw some light upon the origin of the post-natal loss of weight and the nature of the phenomena which succeed it.

The infants born at "The Queen's Home" are fed by the mother when this is feasible, six hours after birth and thereafter every four hours. A little water is given if needed, and milk diluted to one-fourth if the mother's milk is insufficient.

The data enumerated in this as in the preceding article concern only those infants which are the fruit of confinements which were, so far as could be ascertained, normal.

2. THE POST-NATAL LOSS OF WEIGHT

The following tables (1 and 2) exhibit the relationship between the post-natal loss of weight and the length of the period of gestation, the length of the period being estimated from the onset of the last menstruation and those cases excluded in which the infant weighed less at birth than the average weight of infants born thirty days previously or more than the average of infants born 30 days later.[6] Periods lying between 265 and 275 days in length are recorded as periods of 270 days, periods falling upon the limiting date separating two classes (e.g. 275

[5] T. Brailsford Robertson: American Journ. of Physiology.

[6] Cf. the preceding article of this series: *loc. cit.*

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days) being included in both classes (e.g. the 270 and 280 day classes).

TABLE 1

Males

PERIOD OF GESTATION IN DAYS	NUMBER OF INFANTS	AVERAGE WEIGHT IN OUNCES	AVERAGE WEIGHT OF SAME INFANTS AT BIRTH	AVERAGE LOSS (−) OR GAIN (+) OF WEIGHT IN OUNCES
260	5	106	109	−3
270	9	114	119	−5
280	20	119	125	−6
290	14	137	141	−4

TABLE 2

Females

PERIOD OF GESTATION IN DAYS	NUMBER OF INFANTS	AVERAGE WEIGHT IN OUNCES	AVERAGE WEIGHT OF SAME INFANTS AT BIRTH	AVERAGE LOSS (−) OR GAIN (+) OF WEIGHT IN OUNCES
260	3	98	97	+1
270	9	110	110	±0
280	28	118	119	−1
290	21	119	125	−6
300	6	119	122	−3
310	2	127	132	−5

The average weights of the above infants at birth differ somewhat from those recorded in my previous communication because the number of infants of any one class is so small that their average weight at birth does not represent the true average for the class.

It will be seen that these data display no very clear correlation between the magnitude of the post-natal loss of weight and the length of the period of gestation preceding birth although there appears to be a certain tendency for infants born at the later periods to lose more heavily than those born after the briefer periods of gestation. This, as we shall see, is attributable to the fact that the infants born at the later periods are heavier and therefore larger than the infants born at the earlier periods.

The following tables (3 and 4) exhibit the relationship between the post-natal loss of weight and the weight of the infant at birth, all infants weighing for example, between 115 and 125

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ounces at birth being regarded as having weighed 120 ounces at birth. Infants of which the weight at birth fell upon a limiting weight separating two weight-classes (e.g. 115 ounces) being included in both classes (e.g. the 110 ounce and 120 ounce classes).

TABLE 3

Males

WEIGHT AT BIRTH IN OUNCES	NUMBER OF INFANTS	WEIGHT AT ONE WEEK AFTER BIRTH	LOSS (−) OR GAIN (+) IN OUNCES
80	1	91	+11
90	3	86	− 4
100	6	104	+ 4
110	10	108	− 2
120	12	117	− 3
130	9	128	− 2
140	12	132	− 8
150	3	144	− 6
160	3	150	−10
170	1	160	−10

TABLE 4

Females

WEIGHT AT BIRTH IN OUNCES	NUMBER OF INFANTS	WEIGHT AT ONE WEEK AFTER BIRTH	LOSS (−) OR GAIN (+) IN OUNCES
80	3	77	− 3
90	5	94	+ 4
100	7	105	+ 5
110	17	108	− 2
120	19	118	− 2
130	17	125	− 5
140	6	129	−11
150	2	138	−12

It will be seen that there is a very clear correlation between the magnitude of the post-natal loss of weight and the weight (and, therefore, presumably the *size*) of the infant at birth. The infants weighing over 130 ounces at birth suffer especially severely, while infants weighing less than 110 ounces at birth, so far from losing weight during the first week after birth, fre-

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quently gain considerably. From this it would appear legitimate to infer that *mechanical shock during delivery* is an important factor in determining the post-natal loss of weight.

The average weight at one week after birth of all the male infants (=57) born after periods of gestation lying between 245 and 325 days[7] was 121.4 ounces; the average weight of South Australian male infants at birth[8] is 127.3 ounces. Hence the average observed loss of weight during the first week after birth of South Australian male infants is 5.9 ounces or 4.6 per cent of their weight at birth.

The average weight at one week after birth of all the female infants (=79) born after periods of gestation lying between 235 and 325 days[9] was 115.0 ounces; the average weight of South Australian female infants at birth is 121.2 ounces. Hence the average observed loss of weight during the first week after birth of South Australian female infants is 6.2 ounces or 5.4 per cent of their weight at birth.

From these figures it would appear that the growth of female infants is more retarded by birth than that of male infants. That this is not really the case, however, is shown by the following considerations:

It must be recollected that the above figures do not represent the whole of the loss of weight due to birth. At the time of birth the infant is growing rapidly and if it were not for the shock, nutritional and mechanical, of birth the infant would *increase* considerably in weight during the first week of post-natal life. The *observed* loss of weight is therefore not the *actual* loss of weight. We may estimate the actual loss of weight to within a very close approximation, by the following method:

In the preceding article of this series I have shown that the

[7] Thus excluding the periods of gestation eliminated by Chauvenet's criterion as being probably pathological or otherwise abnormal. Cf. preceding article: *loc. cit.*

[8] Cf. the preceding article of this series: *loc. cit.*

[9] Thus excluding the periods of gestation eliminated by Chauvenet's criterion as being probably pathological or otherwise abnormal. Cf. preceding article: *loc. cit.*

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latter part of the pre-natal and the first 9 months of the post-natal growth of South Australian male infants may be represented very accurately by the formula:

$$\log_{10}\frac{x}{341.5-x} = 0{,}136\,(t-1.66) \quad \ldots\ldots\ldots\ldots (1)$$

where x is the weight of the infant and t is the time, measured in months of 30 days, which has elapsed since birth.[10] Now at one week $t = \frac{7}{30} = 0.23$. Hence by putting $t = 0.23$ in the above formula we can estimate what South Australian male infants would weigh at one week after birth if it were not for the retardation of growth and loss of weight due to birth. In this way we find that at one week after birth South Australian males *should* weigh 133.1 ounces. They actually do weigh, as we have seen, 121.4 ounces. The *actual* loss due to the nutritional and mechanical shock of birth is therefore 11.7 ounces or 9.2 per cent of the weight at birth.

The formula which similarly represents the growth of South Australian female infants is:

$$\log_{10}\frac{x}{350-x} = 0.111\,(t-2.47) \quad \ldots\ldots\ldots\ldots (2)$$

and from it we find that at one week after birth South Australian females *should* weigh 126.2 ounces. They actually do weigh 115.0 ounces. The *actual* loss due to birth is therefore 11.2 ounces, which is 9.2 per cent of the weight at birth. Hence males and females suffer an *equal* retardation of growth as a result of the nutritional and mechanical shock of birth.

Since heavy infants suffer a greater post-natal loss of weight than light infants we should expect the *variability* in weight of infants to *decrease* during the first week succeeding birth, since the heavier infants tend to approximate more closely in weight to the light infants. This could only be the case, however, if

[10] Weights at periods antedating birth may of course be estimated by substituting negative values of t in the above formula equal in magnitude to the number (or fraction) of months by which the given periods antedate birth.

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the variability of the loss itself were equal to, less than, or not greatly in excess of the variability in weight of the infants, for otherwise a very highly variable effect of the shock of birth might conceivably lead to an *increase* in the variability of the infants.

The variability of any quantity determined in a series of observations is measured in terms of the "standard deviation" and the *percentage* variability is given by the percentage ratio of the standard deviation to the mean magnitude of the quantity. The percentage variability is the maximum deviation from the mean which 68.27 per cent of the measurements may be expected to display.[11] .

As I have shown in the preceding article the "standard deviation" of the weights of South Australian male infants at birth is 18.2 ounces and the variability is 14.3 per cent. I find that the standard deviation of the weights of South Australian male infants at one week after birth is 17.9 ounces and the variability is 14.7 per cent. The standard deviation of the weights of South Australian female infants at birth is 17.6 ounces and the variability is 14.5 per cent; while at one week after birth the standard deviation is 13.6 ounces and the variability is 11.9 per cent.

It is evident that, as might be anticipated, the post-natal loss of weight is accompanied by a decrease in the variability of the weight of *female* infants. In male however, infants, no such decrease but on the contrary a slight *increase* in variability accompanies the post-natal loss of weight. Evidently, therefore, the *variability of the effect of birth upon males is very considerably greater than the variability of its effect upon females.* The greater variability of the effect of adverse influences upon male infants is very probably correlated with the very much greater infantile mortality which prevails among males than among females,[12] since a greater variability of effect implies a more frequent overstepping of physiological limits.

[11] Cf. C. B. Davenport: Statistical Methods, 2d ed., New York, 1904, p. 16.

[12] According to the Commonwealth Statistician's Official Year Book for 1914, pp. 145 and 172 the ratio of the male to the female death-rate in Australia for death occurring less than 1 week after birth is 139 to 100.

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3. THE GAIN IN WEIGHT DURING THE SECOND AND SUBSEQUENT WEEKS AFTER BIRTH

During the second week of post-natal life there is a marked gain in weight which not only makes good the loss of weight during the first week, but considerably exceeds it. The average weight at two weeks after birth of all of the male infants (=203) born after periods of gestation lying between 245 and 325 days was 130.6 ounces; hence (cf. above) the average gain over the weight at birth at the end of two weeks of post-natal life is 3.3 ounces. The average weight at two weeks after birth of all of the female infants (=233) born after periods of gestation lying between 235 and 325 days was 124.2 ounces, the average gain over the weight at birth being 3.1 ounces.

These gains, however, although they more than serve to make up the *observed* losses of weight due to birth, do not entirely compensate for the *actual* loss of weight due to birth (i.e., the observed loss plus the *gain* in weight which the infant would have displayed had it not been for the retardation due to growth). This may be shown by putting $t = 0.47$ (= two weeks) in the formulae (1) and (2) and computing with their aid the weights which the infants should display at two weeks after birth. In this way we find that at two weeks after birth South Australian male infants *should* weigh 139.3 ounces, while they actually *do* weigh 130.6 ounces. Hence the retardation of growth due to birth is, after two weeks of extrauterine growth, 8.7 ounces or 6.8 per cent of the weight at birth. Similarly we find that at two weeks after birth South Australian female infants *should* weigh 131.2 ounces, whereas they actually *do* weigh 124.2 ounces. Hence the retardation of growth due to birth is, after two weeks of extrauterine growth, 7.0 ounces or 5.8 per cent of the weight at birth.

Although the gain in weight during the second week of extrauterine life is not sufficient to entirely make up the loss due to birth, it will be noted that it *partially* does so, for the loss of weight in males due to birth is 11.7 ounces at one week and only 8.7 ounces at two weeks. In other words the effect of birth does

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not result in a *permanent* subnormality in the weight of the infant because the loss of weight due to birth is made up by a *compensatory overgrowth* (i.e., growth in excess of the normal increment corresponding to the given age and period) which amounts during the second week, in males, to 3.0 ounces. This compensatory overgrowth is even more pronounced in females, amounting to no less than 4.2 ounces during the second week of extrauterine life. Evidently females recover more rapidly than males from the inhibitive effects of birth upon their growth, a fact which doubtless is correlated with the greater tolerance of adverse conditions and the lesser variability of the post-natal loss which female infants display.

The compensatory acceleration of growth during the second week may be shown in another way. From equation (1) it may readily be computed that between the end of the first and the end of the second weeks of extrauterine life the increment of weight in males, if their growth proceeded at the normal velocity indicated by preceding and succeeding weights, should be 6.2 ounces. The observed increment is no less than 9.2 ounces. Similarly the increment during the second week in females should be 5.0 ounces and actually is 9.2 ounces. Evidently the rate of growth in males is accelerated 48 per cent and that of females no less than 84 per cent.

By the end of the first month of extrauterine life this compensatory process has entirely made good the loss of weight due to birth, so that at one month the observed weight of male infants is "normal," that is to say, lies exactly upon the continuous curved line which represents preceding and succeeding growth.[13] In the case of South Australian female infants there would appear, from my previously-published data, to be even over-compensation, since the observed mean weight at one month is decidedly "supernormal."

From these facts it would appear that the normal weight of a growing organism at any given age represents a true dynamic equilibrium, any disturbance of which is rectified by internal regulation, just as a gyroscope restores equilibrium to a mass

[13] Cf. preceding article of this series: Amer. Journ. of Physiology.

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which has been displaced from its "normal" position through the action of an external force. If the induced displacement be too great the inertia of the gyroscope may be insufficient to restore the mass to its original position and analogously we may infer that if the induced sub- or super-normality of the weight of an organism, induced by the action of an adverse environment, exceeds a certain "physiological limit," the internal regulatory processes may be impotent to retrieve the damage to the economy of the organism.

This "internal regulation" of the process of tissue growth is also very well illustrated by the phenomena of tissue-regeneration whether following a wound, when the neighboring tissues are especially stimulated to growth, or following a more generally dispersed loss of tissue-material, as in the marked nitrogen-retention following the wasting of fever or of starvation. The remarkable acceleration of tissue-accretion which succeeds partial starvation upon returning to a full diet is very well illustrated by the following experiment:

Eight young white mice, varying in age between 52 and 123 days, which had hitherto been permitted free access to food and water, were found to weigh an average of 19.0 grams. They were then deprived of food for 27 hours and of water for the last four hours of this period. On again weighing, their average weight was found to be only 15.3 grams. The loss due to 27 hours of starvation was therefore 3.7 grams or 20 per cent of their original weight. They were now given free access to rolled barley, dried bread and water. One hour later they were weighed again and it was found that one-fourth (25 per cent) of the above loss had already been made good; this represents, doubtless, the weight of the contents of the alimentary canal after a full meal. After 21 hours no less than 87 per cent of the loss had been made good, the average weight being now 18.5 grams. Had this rate of increment of weight been continued these mice, already two-thirds grown, would have doubled their weight in five or six days!

This phenomenon of internal compensation is, of course, entirely in harmony with the view which I have expressed else-

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where,[14] that the growth of an organism is regulated by and is the expression of a series of underlying chemical reactions which are of such a nature (autocatalytic) that they produce their own catalysors.

The variability of the weight of male infants at two weeks of age is 14.0 per cent, while that of females is 14.3 per cent. Evidently the extent of the compensatory overgrowth is just as variable, in both sexes, as the weight at birth, i.e., as the preceding "normal" growth. Hence the diminished variability of female infants, which results from the post-natal loss of weight during the first week, is lost during the succeeding week owing to the variability of the normal and compensatory increments during that period.

4. SUMMARY

1. Data are presented concerning the post-natal loss of weight and the compensatory acceleration of growth which succeeds it in South Australian infants, the fruit of normal confinements.

2. There is no decided correlation between the magnitude of the post-natal loss of weight during the first week of extra-uterine life and the length of the period of gestation which preceded birth. There is, however, some tendency for the infants which are born after longer periods of gestation to lose somewhat more heavily than those which are born after briefer periods of gestation. This is because the latter infants are lighter and smaller.

3. There is a very clear correlation between the magnitude of the post-natal loss of weight and the weight (and therefore size) of the infant at birth. Infants weighing over 130 ounces (3680 grams) at birth suffer especially severely, while infants weighing less than 110 ounces (3120 grams), so far from losing weight during the first week after birth, frequently gain considerably. From this it would appear legitimate to infer that *mechanical shock during delivery* is an important, although not the only factor in determining the post-natal loss of weight.

[14] T. Brailsford Robertson: Arch. f. Entwicklungsmech., 25 (1908), p. 581; 26 (1908), p. 108; 37 (1913), p. 497.

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4. Male and female infants suffer an equal retardation of growth due to birth, the actual loss of weight (= observed loss plus gain which would otherwise have been made) after one week being 9.2 per cent of the weight at birth.

5. The variability of the effect of birth upon males is very considerably greater than the variability of its effect upon females. This is probably correlated with the greater infantile mortality of males during the first weeks of extrauterine life, since a greater variability of effect implies a more frequent overstepping of "physiological limits."

6. The post-natal loss of weight is followed by a compensatory acceleration of growth which is more pronounced in females than in males. By the end of the second week of extrauterine life 48 per cent of the loss in males and 84 per cent of the loss in females has been made good by means of compensatory overgrowth. By the end of the first month the entire loss has been made good.

7. The normal weight of a growing organism at any given age represents a true dynamic equilibrium, any disturbance of which is rectified by internal compensation.

8. The following is a summary of the quantitative data reported herein:

South Australian Infants

	MALES		FEMALES	
	Weight in ounces	Variability	Weight in ounces	Variability
		per cent		*per cent*
At birth	127.3	14.3	121.2	14.5
At 1 week	121.4	14.7	115.0	11.9
At 2 weeks	130.6	14.0	124.2	14.3

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THE VASCULAR TONE AND THE DISTRIBUTION OF THE BLOOD IN SURGICAL SHOCK

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INTRODUCTION

We present in this paper a study of the vascular tone and of the distribution of the blood in the condition known as "surgical shock." There is no explicit definition of this term, but for practical purposes in connection with the present investigation, it may be assumed to mean a low arterial blood pressure of such duration that recovery is impossible. Our observations, on dogs, were continued until death intervened. We had therefore the condition as defined. It is apparent to anyone who has studied surgical shock that the condition is a complex one, and it follows that the several factors concerned may establish themselves independently of one another or in varying sequence. Our data clearly support that part of the conception advanced by Henderson[1] that shock is associated with loss of venous tone. This loss of venous tone may initiate a train of events fatal in their outcome, such as the failure of the heart to fill in diastole (Henderson), but the significance of the loss of venous tone relative to other possible factors contributing to shock is beyond the scope of the present paper.

EXPERIMENTAL

Of the numerous experiments performed in approaching the general subject of surgical shock, we desire to emphasize three types particularly, (a) observations of the vena caval and portal

[1] Henderson: American Journal of Physiology, 1908, xxi, 155.

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venous pressures in conjunction with the arterial pressure; (b) observations of the changes in the weight of an isolated loop of gut and (c) observations on the perfusion rate in isolated vascular beds.

(a). *Observations on the venous pressure.* In a few of the experiments, the anaesthetic was morphia and chloretone; in the others morphia and ether. Effort was made to maintain anaesthesia uniformly. Shock was hastened in some cases by exposure of the viscera to air, and in a few cases by manipulation of the exposed viscera, but in general the plan followed was to allow the animal to lie undisturbed after the operation, except for the procedures incidental to the regular observations of the venous pressure. The latter was accomplished by passing a long cannula filled with sodium citrate solution toward the vena cava by way of the external jugular or femoral vein; or into the portal vein by way of one of the branches.

The *vena cava pressure* was observed in thirteen experiments. They all showed a progressive fall in pressure. As contrasted with changes in the portal pressure these experiments exhibited a rather steady fall in venous pressure which was not associated with changes which occurred in the descent of the arterial pressure curve.

The *portal venous pressure* was observed in seven experiments. The pressure falls much less regularly in this system than it does in the vena cava as noted above. It sometimes shows a progressive fall which parallels an even drop in arterial pressure but in general it is quite irregular, in the earlier stages of the experiment rising and falling with the fall and rise of the arterial pressure. Indeed in the majority of these experiments on the portal pressure we felt that we could roughly determine the point of onset of shock by noting the time at which the inverse relationship between the portal and arterial pressure changed to a parallel relationship. Again it may maintain itself for several hours quite independently of the arterial pressure. But sooner or later it falls and this fall in such instances is indicative of approaching death. We incline to take the view from these experiments therefore that the vascular

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changes in shock are chiefly associated with the splanchnic area.

In the experiments such as those described in which graphic records of the venous pressure were obtained there was observed a terminal rise of venous pressure coincident with the death of the animal. This rise is presumably dependent on the ultimate failure of the arterial tone. Mann[2] who has recently reported the observation that the femoral venous pressure is low in shock, noted that in this condition, section of the sciatic nerve produced "an immediate and decided increase in the pressure of the femoral vein." This may therefore be regarded as contributing evidence that arterial tone is at least not wholly lost in shock.

The venous pressure follows the downward course well recognized in the case of the arterial pressure. The evidence advanced by Porter[3] and by Seelig and Lyon[4] that the peripheral arterial resistance is increased in shock in conjunction with these results strongly suggests, as predicated by Henderson, that the blood is stagnated in the dilated veins. This idea is further supported by the observation that the smaller mesenteric veins stand out conspicuously, a fact also noted by Mann. In an effort to illuminate this point, we placed an isolated loop of gut with nerves and vascular supply intact on a balance and observed the results.

(b) *Observation on the weight of a loop of gut.* In two such experiments the anaesthesia was morphia and ether. A loop of the small intestine about 30 cm. long was isolated and wrapped loosely in rubber tissue. The abdominal wall was closed sufficiently to hold back the rest of the viscera, and the animal was suspended vertically. The stalk of the loop of gut permitted the latter to extend far enough to lie on the pan of a delicate balance which was counter-weighted and described its movements on a smoked drum. The stalk of gut, unprotected by rubber tissue was kept moist and soft with an occasional spray

[2] Mann: Johns Hopkins Hospital Bulletin, 1914, xxv, 205.
[3] Porter and Quinby: American Journal of Physiology, 1908, xx, 500.
[4] Seelig and Lyon: Journal American Medical Association, 1908, lii, 45.

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of salt solution. In order that the stalk should not be under tension as the loop of gut changed in weight the counter-weight was frequently altered to maintain a small excursion of the recording lever.

TABLE 1

Heart-rate, respiratory rate, arterial blood pressure and weight of loop of gut (relative) in surgical shock

TIME	HEART-RATE	RESPIRATORY RATE	ARTERIAL PRESSURE	WEIGHT OF GUT (RELATIVE-)*
11.00	60		70	19.0
15	60	28	70	19.0
30	60	28	76	17.5
45	92	22	96	13.5
12.00	122	20	104	9.0
15	134	18	112	10.5
30	134	20	108	6.0
45	164	20	110	4.0
1.00	170	18	100	5.0
15	174	16	110	2.5
30	172	14	114	2.0
45	176	16	116	1.25
2.00	170	16	116	1.0
15	166	16	118	1.0
30	170	14	120	0.5
45	176	16	124	2.0
3.00	186	20	112	7.0
15				4.5
30	188	13	120	5.5
45	188	12	118	6.0
4.00	188	12	120	14.0
15	160	36	114	14.0
30	170	40	100	15.0
45	170	36	92	15.5
5.00	142	56	84	18.5
15	134	50	70	20.0
30	124	42	60	19.5
45	120	20	45	20.5

* Figures used represent movement of recording lever in millimeters.

In the first experiment (table 1), the weight values for the loop of gut are relative only. The loop was little heavier at the end than at the beginning of the experiment. The vessels at the beginning may have been paralysed as the result of manipulation. However this may be, the gut decreased steadily in

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weight for four hours. The arterial pressure rose sharply for two hours and then more slowly for six hours. Toward the end of the latter period the weight of gut varied considerably, and then began a progressive increase which roughly paralled the fall in arterial pressure.

In the second experiment (fig. 1) the plotted curve representing the changes in weight of the loop of gut gives these changes in absolute value. It will be noted that the experiment

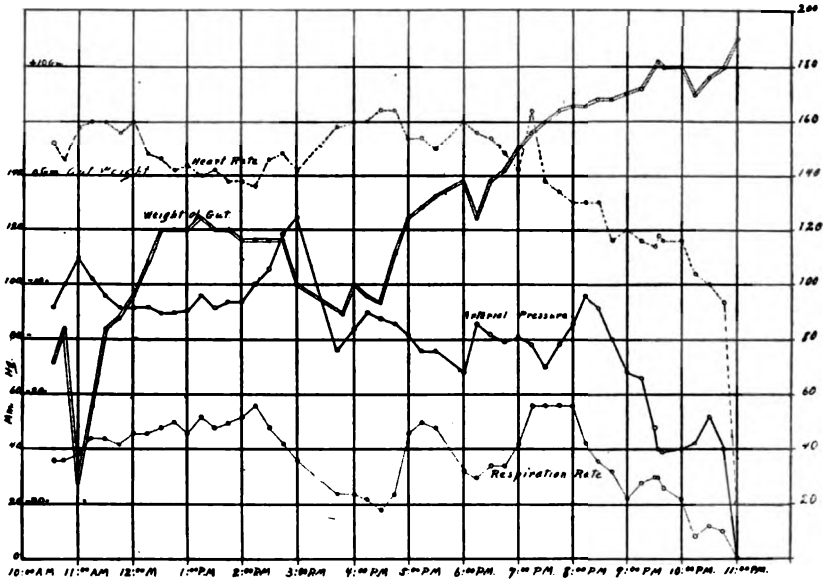


Fig. 1. To show the change in weight of an isolated loop of gut with nerves and blood supply intact in surgical shock. The divisions of the abscissae represent time in hours. The divisions of the ordinates represent (a) 20 mm. Hg. for arterial pressure, (b) 20 heart beats and respiratory movements and (c) 5 gm. for changes in weight of the loop of gut.

lasted over a period of twelve and one-half hours, during which time the loop of gut increased in weight over 30 grams. In the first four and one-half hours the arterial pressure was well maintained, although the loop of gut had increased in weight. At the end of this period the loop of gut lost in weight at a time roughly coincident with the break in the arterial pressure. Thereafter the weight of gut steadily increased while the arterial pressure fell until the end.

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Objection may be offered to the conclusions drawn from such experiments on the ground that an oedematous condition of the tissues might be a cause of the increase in weight observed. This objection cannot be entirely excluded but the general trend of the curves and the appearance of the intestinal loop at the end of the experiments were such as to indicate that oedema played no part in the increase in weight recorded.

The pulse and respiratory rates recorded appear unrelated to the onset of the terminal changes in the arterial and venous pressures, as noted by previous observers. In the curve described by the changes in weight of the loop of gut, a progressive fall precedes the break in arterial pressure, a circumstance which suggests a final effort on the part of the venous portal system to meet the approaching crisis. When this system fails, the arterial pressure begins to fall in spite of an increased heart rate, and the weight of gut increases indicating stagnation of the circulating blood. This stagnation is further indicated by the markedly "venous" color of the shed blood, and by an increased respiratory effort.

These experiments indicate that there is vascular dilatation in the splanchnic area. The results of Porter and of Seelig and Lyon, above alluded to, by exclusion would place this dilatation on the venous side. Porter based his conclusions on the percentile rise and fall of arterial pressure following stimulation of sensory nerves. Seelig and Lyon observed the rate of outflow from the femoral vein before and after section of the sciatic nerve comparing the outflow on one side before shock, with that of the other after shock. Quite recently Seelig and Joseph[5] have demonstrated that if in a rabbit, which has previously had the vascular nerves to one ear destroyed and which is thrown into shock, the arterial pressure be raised by occluding the abdominal aorta the operated ear becomes engorged while the unoperated ear remains normal in appearance. If, after this control, the second ear was operated and the arterial pressure raised both ears become engorged. The conclusion is

[5] Seelig and Joseph: Proceedings Society for Experimental Biology and Medicine, 1914, xii, 49.

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obvious that the vascular tone in the second ear was maintained even in shock.

(c) *Observations on the perfusion rate in isolated vascular beds.* We sought with a technical procedure not hitherto employed to investigate the condition of arterial tone in shock. Our results confirm the findings of others on this point and call for brief mention only. It seemed to us possible that, if the pressure was maintained constant, the rate of flow through a given vascular area might be greater in shock than before the condition was established. It was however necessary to choose areas in which the blood flow should be normal except during the observations. This requirement was established in the case of the hind leg, the kidneys and that portion of the large intestine supplied by the inferior mesenteric artery. For the kidneys inflow and outflow cannulae were laid in the aorta and vena cava distal to the renal branches all minor vessels being tied. Loose ligatures placed above the renal branches permitted the isolation of the organs during the perfusion. For the intestine the inflow was managed in a like manner the outflow being received from a vein caudal to the area under investigation. The loose ligature for the vein in this case was laid about the portal proximal to the area to be isolated. For the leg the femoral artery and vein of the opposite side received the cannulae, other vascular branches being tied, and the loose ligatures for use during isolation were placed above the aortic and vena caval bifurcations. In all ten experiments were performed, using both Ringer's solution and defibrinated blood for perfusate. The perfusion pressure was maintained constant. The temperature of the perfusate was likewise maintained constant except in one experiment in which the temperature was allowed to fall as the temperature of the animal fell. Of these experiments six were performed upon the leg and two each on the kidneys and intestine. In but one of these experiments (leg) was the outflow increased after shock was established. In one of the experiments on the intestine the nerves running to the part were severed after shock was established and resulted in a decided increase in the rate of outflow.

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CONCLUSIONS

1. Both the systemic and portal venous pressures fall in shock.

2. The weight of an isolated loop of gut is increased in shock, a fact interpreted to mean loss of local vascular tone. This loss of tone may be arterial or venous or both. Our evidence indicates loss of venous tone which would predicate failure of the veno-pressor mechanism and a stagnation of venous blood.

3. Perfusion of vascular areas, temporarily isolated for observation, shows a decreased rate of flow in shock.

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SOME CHARACTERISTICS OF VASOMOTOR REFLEXES

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The original purpose of the experiments to be described in this paper was to test the effect upon blood-pressure of stimulation applied to each of two afferent paths and to both simultaneously. It was thus closely analogous to the purpose of Camis (1) in his study of the reflex contractions of a selected skeletal muscle. He found that in many instances a more extensive contraction could be produced by stimulating two or three afferent paths at once than could possibly be secured through one alone. A degree of diffuseness and a lack of unity in the motor centres was inferred. It seemed a matter of interest to make similar trials for the vasomotor mechanism; to see whether greater effects upon arterial pressure could be produced through the excitation of a large number of afferent fibres than could be obtained with a smaller number. As the work proceeded other matters, not foreseen at the outset, claimed a share of our attention.

After we had pursued our task for some time we discovered that W. T. Porter, in 1908 (2), had projected a similar study. He had suggested that an investigation of this kind would throw light on the comparative effects of stimulating small and large areas of the skin by temperature changes or otherwise. We wish to give all the credit that is due to Dr. Porter but the lapse of time and the unconsciousness of any invasion of his field, so far as we were at first concerned, appeared to us to warrant the continuance of our work.

Many years ago Grützner and Heidenhain (3) were impressed by their observations that localized stimulation of the skin

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is less efficient in causing pressor reactions than stimulation applied to large areas, even when the former is intense and the latter mild in character. Unfortunately, they did not attend to the fact that they were stimulating receptors instead of nerve-fibres. A mode of stimulation which may seem to be gentle may be in reality highly effective in generating impulses when it is brought to bear upon appropriate end-organs. This is probably the case with blowing upon the skin, a measure often employed by Grützner and Heidenhain. From a purely physical standpoint this is a mild application but biologically it is a potent one since there are specific endings ready to respond to it and to transmit impulses along the afferent channels of the mechanism for temperature regulation.

The salient facts regarding vasomotor phenomena attending the stimulation of afferent nerves may now be recalled. The effect on the blood-pressure varies with the strength of the excitation. With weak shocks there is a fall which becomes gradually more marked as the strength of the stimuli is increased but soon lessens again and is exchanged for a rise when a certain rather definite stimulation strength is exceeded. Martin and Lacey (4), using the quantitative method developed by the former, have found that this "threshold of the pressor reaction" is passed when the value of the shocks is rated at about 280 Z units in terms of the Martin system (5). With additional increase of stimulation the elevation of the blood-pressure becomes more and more marked through a long range. The account given is valid for vagotomized animals.

When we compare the effect of stimulating two nerve-paths at the same time with that of exciting each by itself we have to face a problem of summation. But in the large literature of this subject the reference is usually to the successive application of two or more stimuli at one place (6). The summation with which we had to deal was a simultaneous variety although there must have been present in it the element of successive shocks. The central machinery was played upon by both streams of impulses. When we undertook to observe the influence of weak currents such as would cause a reflex fall of blood-pressure it

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was evident that the interpretation of records must be difficult. Summation of effect might appear as an increased *fall* of pressure or, if the two weak applications should prove equivalent to one much stronger, a reversal of effect might be witnessed or, possibly, there might be no disturbance at all if the pressor and depressor tendencies should chance to balance. These sources of confusion were anticipated but it seemed reasonable to expect that if the stimulation applied to each of the two nerves was sufficiently strong to produce a pressor effect, then the simultaneous employment of two such excitations should give results of a clear character.

It may be suggested that to stimulate two afferent nerves at the same time may be merely equivalent to the stimulation of one of them with shocks of a higher frequency. But Martin and Lacey (4) have found that the interruption of the primary current can be made to take place at widely varying rates without affecting the extent of the vasomotor change resulting from the stimulation of a single afferent path.

Method. The animals used were cats, anesthetized by ether or urethane. The carotid pressure was recorded by a mercury manometer. Different nerves were chosen for stimulation, sometimes symmetrical pairs, sometimes two on the same side of the body, and sometimes two unsymmetrically located but on opposite sides of the axis. Sherrington electrodes, or a modified form devised by Martin, were placed on the nerves and every precaution against needless exposure was taken.

The primary current (usually 0.1 ampere) supplied to Martin's calibrated coil was interrupted by the vulcanite-mercury key, described in other papers (7). The key was worked by a crank on the shaft from a large pulley belted to an electric motor. Both makes and breaks were allowed to take effect in order to minimize polarization.

The question may be asked whether anything akin to polarization actually occurs, under the best conditions, when a segment of nerve is subjected to an alternating current for a considerable time. We believe that under the conditions of our experiments there is no appreciable impairment of irritability and

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no diminution in the effectiveness of the shocks from any such local cause. Our opinion is based on the following type of experiment. Two pairs of Sherrington electrodes were placed on the same nerve. The peripheral pair were used to apply prolonged stimulation. When the vasomotor response waned the stimulation was brought to bear upon the fresh segment of the nerve between the first point of application and the centre. With comparable strengths of current it was found that the shift did not add to the efficiency of the stimuli.

We will now pass to the routine procedure. By manipulating plugs the secondary currents could be sent through either pair of electrodes or both in series. It will be evident that when the current is sent through segments of two nerves in series, instead of one, an increased resistance is encountered and the stimulating potency at a given point is diminished. This difficulty we overcame by the following arrangement. A Wheatstone bridge could be employed at any moment to determine the resistance of the secondary with either nerve in circuit. The same resistance could then be provided by means of a rheostat and the current made to traverse the box when not directed through the nerve. Thus when one nerve was stimulated the current was tempered by an artificial resistance equal to that of the other nerve and when both were in circuit the resistance was removed.

At the outset we need to know the typical features of the reflex changes in blood-pressure when only one nerve is stimulated. The fall of pressure produced by weak stimulation is mild in degree, rarely over 10 per cent, and transient, recovery taking place in spite of continued excitation. It has been ascribed to a reflex mediated by the vaso-dilators (8). Pressor responses are much more variable. Sometimes the tracing shows a peak and a steady decline within the period of stimulation, as though fatigue of some sort were manifested by the mechanism. Sometimes the record has a plateau character, giving little impression of fatigue and showing a prompt subsidence when stimulation ceases. There is a third possibility, that a plateau formed under the influence of afferent currents

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may be continued with little sagging in the after-period. In such cases the blood-pressure keeps for a long time a new and high level. We have called this a "boosting reaction" and we cannot yet recognize the conditions which favor its occurrence.

A curve given by Sherrington (9) shows a pressor reaction continuing far beyond the period of stimulation, with a slow decline to the original level. Such instances, which we have often seen, suggest the possibility that adrenalin may have been released as a result of the stimulation. But we cannot attribute to adrenalin a rise of pressure which does not at all outlast the stimulation period.

In some of our earlier trials we were able to demonstrate a moderate degree of summation even when we did not employ compensatory resistances. That is to say, a current carried through segments of two nerves in series and tempered by their combined resistance was more effective than when applied to only one nerve even though in this latter case the lowered resistance insured a considerable increase of stimulation. Naturally enough, we were able to confirm the fact of summation when the omission of either nerve from the circuit was compensated for by introducing an appropriate resistance. But it is fair to say that the extent of possible summation, when two nerves are stimulated instead of one, is quite limited. (Figures.)

Another matter eventually proved to be of rather more interest than the primary question of summation. This was the possibility of establishing a pressor reaction by stimulating one nerve and then prolonging or even intensifying it by shifting the exciting current to another nerve. Again and again we found that we could overcome the flagging of blood-pressure when the first application ceased to be effective by introducing a second in place of the first, that is, by substitution instead of summation. The work of Forbes (10) may be referred to as throwing light upon this phenomenon. His studies, like those of Camis, were upon reflex contractions of skeletal muscles.

Forbes showed that when the reflex response of a single muscle can no longer be secured by stimulating the path which originally produced it, a renewed contraction may be counted on if

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another afferent path is substituted. The inference is that the fatigue which is manifested when the reflex at first disappears is due to the blocking of a line of approach to the centre. Presumably it is a case of rising synaptic resistance. It is assumed that the nerve-cells which are conceived to form the centre



Fig. 1. Summation of depressor effects. Nos. 11 and 12 show the slight reaction obtained by stimulating each of two nerves, No. 13 the more marked response when both are stimulated at once, the shocks remaining of the same intensity as before.

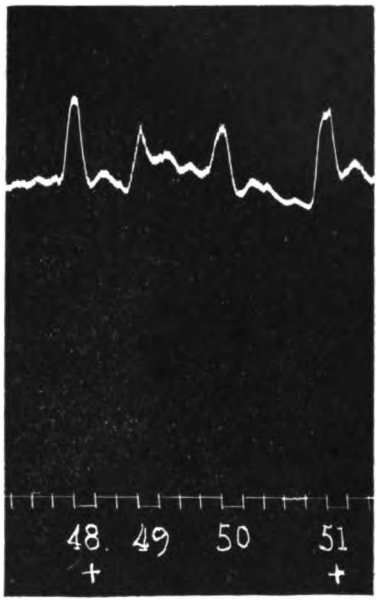


Fig. 2. Summation of pressor effects. Nos. 48 and 51 show the effect of strongly stimulating two nerves at once, Nos. 49 and 50 record the individual responses to the stimulation of each of the two nerves alone. The stimuli were of equivalent strength throughout.

and the efferent paths are the same, first and last. Therefore, the primary fatigue cannot be of the motor apparatus nor of the presiding neurons; it must be sought on the afferent side.

The same conclusion is suggested when a failing pressor reaction is reinforced by shifting the stimulation to a second nerve.

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Here again, if we assume that the same muscular elements are concerned each time the pressure is raised, and that the centre has such a degree of unity that all its cells are involved in each pressor reflex, we shall be led to believe that fatigue of particular approaches is to be reckoned with. An alternative view will be presented later.

One characteristic of the vasomotor mechanism is the rapid recovery from fatigue. If we evoke a pressor response which nearly passes away in the course of 30 seconds or a minute of continued stimulation, we can duplicate it with entire success after an interval of rest equal to, or even shorter than, these periods. It is to be noted that continuous stimulation after the blood-pressure has fallen back to its original level is not likely to revive the pressor reaction. Yet, after an intermission of a minute or less, the resumption of stimulation (the intensity being as before) may reproduce the original elevation of pressure. In other words, the continued excitation has prevented recovery from fatigue at the centre even after it has ceased to produce peripheral effects. Quick fatigue and quick recuperation are more naturally associated with synapses than with other features of the system.

Noting that a very short period of rest restores the capacity for full pressor reactions, we were interested to find out whether the alternate stimulation of two afferent paths would give a better sustained elevation of blood-pressure than the continued stimulation of one for a long period. The shifting was sometimes effected once in 30 seconds, sometimes once a minute, and occasionally at longer intervals. The resistances of the nerves were known and, unless the two were nearly equal, the stimulation of either one was through a circuit containing a resistance equal to that of the other nerve.

Another method of assuring comparable stimulation of two paths was also employed. This was to find for the two nerves two positions of the secondary coil with which equal pressor effects could be secured and then to use with each nerve the appropriate strength of stimulation.

As we accumulated results we viewed them with increasing

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dissatisfaction. It appeared in some cases that the adoption of a new path of approach did not at all reinforce the failing reaction. In other instances the reinforcement was most striking. It was only when we reviewed a large mass of these data that they began to assort themselves in an orderly way. All the observations were found to be consistent with the following principle: when a pressor effect, secured through stimulation of given path, declines, little is gained by transferring the application to a *neighboring* nerve, but a renewal of the pressor reaction is generally to be counted upon when the stimulation is shifted to a *remote* part of the body. The "remote" locality may be symmetrical to the first or far removed from it on the same side. When the choice of nerves is fortunate a judiciously managed alternation of the two may hold the blood-pressure at a well sustained elevation for many minutes.

An experiment may be cited to illustrate the facts just stated. A cat under urethane was vagotomized and two nerves of the right leg were prepared. They were the popliteal and peroneal. The left peroneal was also made ready for stimulation. After preliminary trials to find a suitable strength of current the right popliteal was strongly stimulated for 6 minutes. The pressor plateau was well sustained. After 2 minutes rest the two nerves in the right leg were subjected to alternate stimulation, shifting after each 90 seconds, for 7½ minutes. The popliteal received the same stimulation as before; the peroneal a current previously determined to give a pressor response of the same order as its fellow. The plateau produced in the record by alternate stimulation nowhere exceeded the height of that secured from the popliteal alone and repeatedly sagged when the change from one nerve to the other was made.

For comparison, the left peroneal was stimulated for 6 minutes. This gave a considerable rise of pressure at first and then a decline to a plateau which was held to the end. After a rest of one minute this nerve and its companion on the right—a "remote nerve"—were stimulated alternately, changing once in 90 seconds. The height of the previous plateau was twice surpassed in 7½ minutes while each shift brought reinforcement, never sagging.

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How shall we interpret the facts? There seem to be two possibilities. (1) If the vasomotor centre is assumed to have strict unity the reinforcement obtained through employing a fresh afferent path of excitation in place of one which has ceased to be effective may be explained along the lines of Forbes' conception. But if remote nerves must be selected to secure positive results we shall have to conclude that neighboring nerves utilize a common approach to the centre. (2) We may incline to the view of Camis, assuming that the centre is not strictly integral in action. If this is the case it may be inferred that the stimulation of two nerves near together affects the vessels in a field which is but little different from that which either one commands. Fatigue may really be on the side of the effectors. The renewed effect produced by stimulating a distant nerve may thus be due to the contraction of blood-vessels not previously involved. But if the vasomotor centre were much given to partial reactions we might well look for strongly summated effects when the stimulation of two nerves remote from one another was simultaneous.

SUMMARY

1. Stimulation of two afferent paths at the same time has often a more marked vasomotor effect than the stimulation of either path alone with an equivalent strength of current. The degree of summation is only moderate.

2. When a pressor reaction, secured by stimulating a given nerve, declines it can often be renewed by shifting the stimulation to a second nerve. The renewal is much more to be relied on when the second nerve is distant from the first than when it is near.

3. The superior reinforcing power of a distant nerve may be accounted for on the theory that its afferent connections with the centre are unimpaired by previous use (the Forbes principle) or we may suppose that it has access to a fraction of the central mechanism not previously stimulated and through this to a fresh set of vessels.

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A STUDY OF THE CAUSES OF RESPIRATORY CHANGE OF HEART RATE

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INTRODUCTORY

Of all the factors the sum total of whose effects results in the respiratory wave of blood pressure, a factor of great interest, and as yet not fully understood, is that of the respiratory change of heart rate. The chief explanations of the change in heart rate may be grouped under two headings, namely *reflex* (Einbrodt, Hering, Luciani) and *automatic* (L. Traube, Fredericq).

These explanations are so pertinent to the matter about to be presented that it seems necessary to restate them and the evidence upon which they were based.

Luciani[1] who still holds to Einbrodt's view, explicitly speaks of the act as reflex, saying that he takes the respiratory change of heart rate "to be the effect of a reflex rhythmical excitation of the bulbar centre of the cardiac vagi during the expiratory acts." From this statement one must suppose that the expiratory act sets up afferent impulses that are reflected back to the heart from the vagal centre, thus causing slowing of rate. A perusal of Einbrodt's own paper does not make this so clear. He may have meant that the activity of the expiratory centre in the bulb influenced the vagal centre, increasing its tone and thus causing slowing of the heart rate.

Both Einbrodt and Luciani are agreed that it is the expiratory act that (reflexly or automatically) increases vagal tone.

Hering[2] based his conclusion upon an experiment in which he observed in the dog respiratory change of heart rate during

[1] Luciani, L.: Human Physiology, London, 1911, i, p. 435.

[2] Hering, E.: Wiener Sitzungsberichte, 1871, lxiv, p. 333.

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artificial inflation of the lungs as well as during normal breathing. The conclusion was that the mechanical stimulation of the pulmonary terminals of afferent vago-sympathetic fibres reflexly stimulated the cardiac accelerator centre, and thus increased heart rate during inspiration.

But L. Traube[3] and Fredericq[4] showed another possibility. The former observed respiratory change of heart rate both in dogs deeply curarized and with sectioned cervical cord, the latter in dogs with patent chest walls and lungs collapsed. The conclusion was that respiratory change of heart rate was brought about by "an automatic rhythm common to the respiratory and cardio-motor centres." The quotation is from Fredericq's[4] classical work, in which much was done to clarify the relations of these centres one to another. Nevertheless Fredericq's statement leaves a certain vagueness as to the locus and mechanism of the stimulus which leads to respiratory change of heart rate. For this reason it is necessary to paraphase his statements and if possible put them in more modern phraseology.

In the first place Fredericq is convinced that respiratory change of heart rate may not be a true reflex; the original stimulus need not arise in the periphery. His experiment shows that beautifully. Its origin must be in the bulb itself. In the medulla there is an *automatic* rhythmical mechanism which is common to the respiratory, the vaso-motor and the cardio-motor centres. It is thus that rhythmical tonus waves, emanating synchronously with the respirations from the vaso-motor centre, may produce respiratory waves in the blood pressure by rhythmically changing the calibre of the blood vessels (true Traube-Hering waves). It is thus that tonus rhythms emanating from the cardio-motor centre synchronously with the rhythmic activity of the respiratory centre may produce respiratory waves of another character, waves of changing heart rate. It is during *expiration* that both the vaso-motor and cardio-motor centres receive their maximum influence from this "rhythme automatique." And so it happens that often no

[3] Traube, L.: Gesammelte Beiträge, 1865, i, p. 390. Quoted from Fredericq.
[4] Fredericq, L.: Archiv de Biologie, 1882, iii, p. 55.

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respiratory wave obtains in the blood pressure. For the action of this "rhythme automatique" upon the circulatory centres have opposing effects. If the vaso-motor centre be more excitable than that of the cardio-motor, the result may be the paradoxical wave of rising pressure during cardiac retardation, of falling pressure during cardiac acceleration. Again if the cardio-motor centre be the more irritable, one observes a respiratory wave of falling pressure with cardiac retardation, of rising pressure with cardiac acceleration, the opposing vaso-motor effect being entirely masked by the greater effect of changing heart rate.

The conceptions of both Luciani and Fredericq are the same then in so far as they attribute *the prime agency leading to change of heart rate to be directly connected with the expiratory act.* The difference in the two authors is that the one regards the phenomenon as a true reflex, the other as an automatic influence, possibly of some intermediary rhythmical process coexisting in the medulla. In more modern literature physiologists are inclined to regard this intermediary process as superfluous. The same effect is had by the possible spreading of the activity of one centre to neighboring centres. This process has long been spoken of as *irradiation.*

Turning to clinical literature one finds views of greater variance. On the whole, however, the question seems to have received slight attention from clinical men. How great the variance of views is is indicated by Mackenzie[5] when he says that many of his fellow practitioners regard respiratory change of heart rate as a pathological symptom of a condition requiring treatment, but that he himself after many years' observation came to regard it as "a youthful type of irregularity," and finally as an indication of "a healthy heart." This author makes no considerable attempt to explain the phenomenon, aside from the statement that "the slow respiration induces an irregular action of the heart due to stimulation of the sino-auricular node. The condition is due to vagus stimulation

[5] Mackenzie, James: Diseases of the Heart, 3rd edition, London, 1913, pp. 55, 184, 187.

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. . . ," and again, "the vagal effect produces the irregularity." From which one is left to infer that vagal centre tonus rises and falls with the respirations. As to a cause of the rise and fall of vagal tonus nothing is said.

NEW OBSERVATIONS

It may be stated at this point that the physiological evidence on the nature of the mechanism producing respiratory change of heart rate, is all in favor of the *automatic* or *irradiational* hypothesis and against the *reflex* hypothesis, so far as that implies origin of stimulation at the periphery.

I. On the mammal

During routine work confirmation of this view is often observed, but rarely does it appear in such clear and irrefutable form as in the record here reproduced.

The record, however, contains more than a refutation of the *reflex* hypothesis of respiratory change of heart rate. *The record contains very clear evidence that it is not the expiratory act but rather the inspiratory act* (*at most the whole respiratory act*) of the respiratory centre that gives rise to the influences leading to change of heart rate. Furthermore the influence is not to increase vagal tone, but to decrease or remove it. Other evidence will be submitted to show that the *expiratory* act neither reflexly nor automatically can be the usual cause of respiratory change of heart rate. And this then is at once the justification not only of its publication but also of the foregoing somewhat detailed exposition of older and well known work.

a. Vagal tone produced by electrical stimulation. The conditions under which the observations were made were somewhat as follows (see fig. 1):

The animal, a dog, is the usual experimental preparation under morphia and ether, tracheotomized with ether bottle attached. A mercury manometer is attached in one carotid. Both vagi have been exposed, one of which, the right, has been divided; the other has been left, as the rest of the animal, intact.

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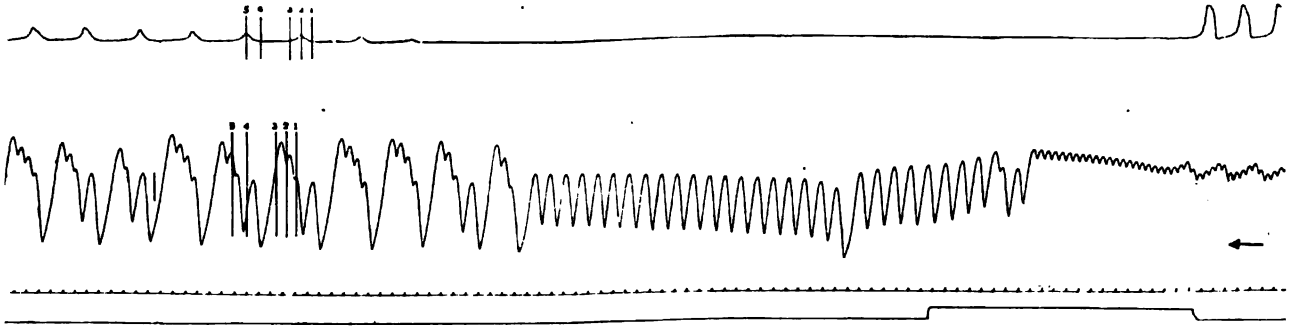


Fig. 1.—From the record of the experiment of May 19, 1914, reduced to ½ of the original. The figure is to be read from right to left. The trace at bottom marks zero blood pressure and also serves as stimulation signal. The next trace is time in seconds. The trace next to the top trace is the blood pressure (mercury manometer) trace. The topmost trace marks the respirations; the upstroke indicates inspiration. Scratch marks on the record show that the writing tip of the respiratory pen stood from 5 to 7 mm. to the right of the writing tip of the blood pressure pen in the original.

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Stimulation of the central stump of the divided vagus gave the remarkable results which are shown in the portion of the kymograph record here reproduced (see fig. 1). One reads the figure from right to left. Scratch marks appearing on the record near the portion which is here exhibited, show that the writing tips of respiratory and blood pressure pens deviated from the vertical from 5 to 7 mm., the respiratory point being to the right of the blood pressure point. This correction is used in placing the scratch marks in the figure, synchronous points being indicated by corresponding numerals. The upstroke of the respiration lever marks the inspiratory movement. In the record the lowest trace marks simultaneously zero pressure and the points of stimulation. The time mark at the top is in seconds. The beginning of the record in the figure (right side) shows the pulse rate to be about 116, the respiratory rate 20. The blood pressure trace shows a well marked respiratory wave. The mean pressure is about 128 mm. Hg.

Faradic stimulation is then applied to the central stump of the divided vagus for a period of about 22 seconds.

The events that follow are:

1. A prompt inhibition of the respiration at the end of the expiratory phase.

2. A slow rise of mean blood pressure for about the first 13 seconds of the stimulation, when a pressure of 140 mm. has been reached.

3. At this point the stimulation begins to affect the cardio-inhibitory centre which, through the intact vagus, slows the heart rate in an unusually smooth and regular fashion. The new rate is about 44 beats per minute.

4. The mean pressure in this last stage at first falls slightly below normal and then remains constant at about 100 mm. Hg.

5. The respiratory movements continue in perfect inhibition for some time after removal of the stimulation; the blood pressure trace with its greatly slowed pulse rate, as well as the respiratory trace, shows no sign of a respiratory wave.

6. This picture continues for some 32 seconds, when the blood pressure trace suddenly shows great waves which turn out to be unmistakable respiratory waves.

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7. *At the time of the appearance of the first of these respiratory waves, no sign of mechanical act of respiration can be found on the respiratory trace.* By the time the second wave appears, the respiration lever shows the smallest indication of respiratory movement. From this point on, the pneumograph movements slowly and gradually become augmented in size until they reach normal dimensions.

8. Now a remarkable thing appears. As the respiratory movement becomes augmented, the size of the respiratory waves just as gradually and slowly become smaller until (it would be too long a trace to reproduce) they are again of the dimension and character of the respiratory waves found in the blood pressure record just before the stimulation was applied to the vagus.

If one compares the respiratory trace (noting scratch marks) with the blood pressure trace carefully, one becomes convinced that in this part of the record the *slowing of the rate occurs during the pause between the expiratory and following inspiratory acts. The acceleration on the other hand begins sharply with the inspiratory act* and continues to the end of the expiratory act.

With the end of expiration the inhibition, or great retardation, again sets in promptly.

The sudden appearance of the respiratory waves upon the smooth blood pressure trace at a point *just before* the respirations are resumed, can only be interpreted as follows:

1. The respiratory centre just recovering from the inhibition produced by faradization is throwing out impulses to the inspiratory mechanism. These impulses are ineffective, but the energy of the active centre is powerful enough (one imagines the activity to arise as rhythmical explosions) to interfere with the exaggerated tone of the vagal centre brought on by the previous electrical excitation.

The inspiratory explosion being over, its action upon the vagal centre ceases and the latter's exaggerated tonus is free to express itself again in the marked cardiac retardation that one observes in the record.

2. As there were, on account of the electrically elicited inhibition of respiratory centres, no actual respiratory movements.

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it is clear that there could have been no intra-thoracic or intra-peritoneal changes of pressure, and hence also no peripheral stimulation of afferent vagal terminals. *The first respiratory blood pressure waves therefore* (as in Fredericq's dog with patent chest walls and collapsed lungs) *must be referred to a central causative agent.*

3. The fact that the respiratory change of heart rate diminishes as the inspiratory movements increase (not shown entirely in the figure), on the other hand, is still additional evidence against Hering's *reflex* hypothesis, namely that mechanical stimulation of afferent terminals in the pulmonary vagi reflexly gives rise to the phenomena of respiratory change of heart rate.

4. Inspecting the section of the record not all shown in figure 1, one notes that it is the inspiratory and not the expiratory movements that are being augmented. The pneumograph lever drops back always to what is clearly a constant expiratory base line. Furthermore the expiratory movements are of the passive kind; there is no evidence that they involve either nervous or muscular energy. The activity of the expiratory centre at this point may therefore be considered as nil.

5. The fact that the respiratory waves gradually diminish with diminishing change of heart rate is clearly explained by supposing that the hyperactivity, or exaggerated tone of the vagal centre, brought on by the electrical excitation, gradually subsided, as in experience it always does. The inspiratory centre thus no longer influences the vagal centre so markedly, not because the inspiratory centre is less active (it apparently is much more active), but because the vagal centre is under less tone and, therefore, has less tone to be removed.

To sum up briefly, the record here reproduced contains new and unmistakable objective proof that the cardio-motor centre may be directly influenced by its neighboring respiratory centre. The evidence of Fredericq which originally led to this belief was obtained upon an animal with open thorax and collapsed lungs. The evidence in the case here reported is obtained upon an animal with chest walls intact, but with the respirations tempo-

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rarily thrown in a state of inhibition, and the cardiac vagal centre simultaneously thrown into a state of increased tone. Rhythmical change of heart rate suddenly appears and directly thereafter a return also of the respiratory movements. The time relations of the blood pressure wave, produced by the rhythmical change of heart rate, and the reviving respiratory movements are such that one concludes that the change of heart rate was initiated by the reviving activity of the respiratory centre before the latter was able to cause respiratory movements. The effect of the activity of the respiratory centre was to remove the vagal tone and thus allow cardiac acceleration, cardiac retardation taking place again during the respiratory pauses.

These time relations also indicate that it is most probably not the activity of the *expiratory* centre that *stimulates* the vagal centre, but rather the activity of the *inspiratory* centre which *depresses* the vagal centre, or possibly excites the hypothetical cardiac accelerator centre.

b. Vagal tone produced by epinephrin. The above case of increased tone of vagal centre was produced by faradization of the vagal nerve. The same effect may be produced by intravenous injection of epinephrin. Here again, as is shown in figure 2, acceleration of heart rate promptly sets in with the beginning of inspiratory movement; retardation of heart rate begins at the end of expiration and continues during the respiratory pause. As in the case of electrical stimulation, so also in the case of epinephrin injection, the preliminary condition to vagatonie is increased blood pressure; the respirations may or may not be arrested. In figure 2 the epinephrin given was not sufficient to interfere with respirations in any noticeable degree. This however may be accomplished by increasing the amount of epinephrin.

Indeed Mr. M. C. Sosman in the course of an investigation which he is carrying out in this laboratory has been able to reproduce all the effects obtained by faradic stimulation (as shown in fig. 1) by epinephrin injection alone. His experiment and results were as follows:

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A dog of about 6 kgm. weight under morphia and ether was given intravenously about 5 mgm. of partially oxidized epinephrin. At once the usual rise of pressure appeared then very marked slowing of heart rate. The blood pressure however remained high in spite of the great slowing of heart rate and the respiration was completely arrested at the end of 70 seconds.

At the end of 118 seconds with the respirations still inhibited rhythmical change of heart rate set in which condition continues for 51 seconds longer, when shallow inspiratory movements begin. The periods of these respiratory movements have the same time as the rhythmical waves of changing heart rate.

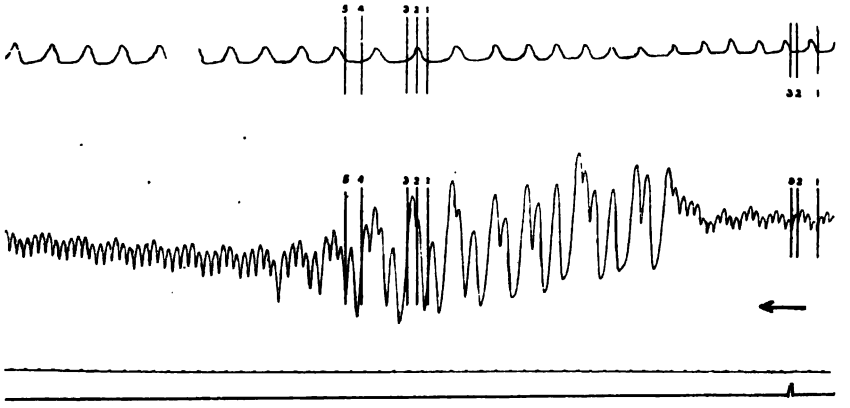


Fig. 2.—From the record of the experiment of April 24, 1914, reduced to ½ of the original. The trace marks are to be read in same order as in figure 1. In this record the scratch marks were all on same vertical. At the signal mark 3 cc. of weak solution of epinephrin was intravenously injected. The dog was under morphia and ether and both vagi were intact.

Here again it appears that the activity of the respiratory centre may effect the vagal centre long before it does the respiratory mechanism itself.

II. On man

The above section has only dealt with the experimental animal. Do the same relations hold between the bulbar centres

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in man? Respiratory change of heart rate in man has been long a matter of observation. The general rule has been that cardiac acceleration accompanies inspiration, while retardation accompanies expiration.

If one asks a patient in whom respiratory change of heart rate is present to hold his breath at the end of a normal expiration, or rather to allow the chest walls to pause in a perfectly passive condition, *the retarded character of the heart rate continues.* This retardation cannot now be attributed to the action of the expiratory centre, for that is in a state of quiescence.

Examination of such a subject leads to only one conclusion, namely that the heart is under the influence of a constant vagal tone which for that condition constitutes the fundamental rate for the heart. *This fundamental rate undergoes change (acceleration) only when the influence of the reviving activity of the inspiratory centre reaches the vagal centre.* The effect is, then, inhibition of the vagal centre, hence inspiratory acceleration.

If the vagal tone be removed by drugs or fever (as Fredericq showed) respiratory change of heart rate drops out, either because the endings of their nerves or the cardio-motor centres themselves have lost their exquisite state of irritability, or inner activity.

It is important to pause at this point to consider other reasons that point against the possibility of the expiratory act producing retardation.

1. In quiet normal breathing it is the inspiratory phase which is the active phase of the movement complex; the expiratory phase is regarded generally as being perfectly passive and requires no muscular tension, no innervation.[6]

2. In the same normal breathing it is the inspiratory act which is inhibited by afferent pulmonary impulses in the vago-sympathetic trunks; the expiratory centre under this condition receives no inhibition—there is no activity to inhibit. On the contrary if further expiration is required, stimulation, not inhibition, of the expiratory centre begins at this point of the respiratory cycle.

[6] See however, Howell, W. H.: Textbook of Physiology, 5th edition, 1913, p. 685.

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3. Furthermore it is an inspiratory not an expiratory act which terminates a period of apnoea.

4. Our general knowledge of the agents that bring on vagotonie, i.e. excitation of the vagal centres, would lead to the view that normal activity of other neighboring centres is not one of them, rather is such activity productive of a depression or removal of vagotonie.[7]

5. Cardiac vagotonie is not specially a rhythmic condition. Our conception of it is a *constant* and not an *intermittent* condition of the vagal centre. In case of respiratory change of heart rate it is more likely, then, that the activity of the inspiratory centre may with its rhythmical action also depress its neighboring cardiac vagal centre, that is, momentarily remove the tonus and *thus rhythmically remove the excessive slowing of the heart rate, hence cause cardiac acceleration.*

6. In his exhaustive work on the accelerator mechanism of the heart, Reid Hunt[8] it will be remembered came to the conclusion that "almost all cases of rapid heart action are due to a diminution of the tonic activity of the cardio-inhibitory centre."

SUMMARY

1. Respiratory change of pulse rate in experimental animals (dogs) is described, consisting of a marked acceleration during the whole of the respiratory movements, and a marked retardation during the pause between the respirations.

2. The blood pressure changes thus produced consist of inspiratory rise lasting to the end of expiration, and a fall lasting during the pause between the respirations.

3. This respiratory wave of blood pressure was produced (a) by electrical stimulation of the central stump of one vagus, the other vagus being left intact; (b) by intravenous injection of epinephrin.

[7] Meltzer, S.: Archiv f. Anat. u. Physiologie, 1883, p. 221. It is here shown that cardiac acceleration accompanies the act of swallowing, which the author explains as an inhibition of vagatonie.

[8] Hunt, Reid: Amer. Journal of Physiology, 1899, ii, 435.

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4. When the "respiratory" waves were produced electrically their appearance on the blood pressure trace was preceded by prolonged respiratory inhibition. Contrary to expectation it was not the respiratory *movements* which were first revived but the respiratory *blood pressure waves themselves*.

5. The time relations of the reviving respiratory movements, which appeared soon after the blood pressure waves made their appearance, were such that one and one conclusion only could be made, namely that it was the activity of the reviving *inspiratory* centre which, though too feeble or meeting too much resistance, to innervate the appropriate respiratory mechanisms to movement, still by some means (irradiation of energy, automatic rhythm, Fredericq) was able to influence its neighboring vagal centre in such a way as to remove from it the excessive tone under which it for some time previously had been laboring. The removal of the vagal tone appears to have been accomplished by an actual depression of the inhibitory centre.

6. Similarly, when cardiac slowing is produced by injection of epinephrin, the time relations of the respiratory waves in the blood pressure to those of the respiratory movements are such as to point again to the activity of the inspiratory centre as being the initial agency in the production of the respiratory wave, and in the same manner, that is by inhibiting the vagal centre.

7. It is further pointed out that in many cases respiratory change of heart rate in man may be likewise explained. The probable cause in such cases is the existence of excessive vagal tone proceeding from a highly sensitive vagal centre. This heightened tone is intermittently depressed or removed during the activity of the inspiratory centre. So soon as the *inspiratory* activity ceases (during expiration or the respiratory pause) the tone of the vagal centre is free again to act causing the familiar expiratory retardation of the heart.

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CONCLUSION

The thesis here presented the author believes is new in so far as

(a) It gives proof, of a different character from that given by previous workers, showing that the cause of respiratory change of heart rate is in the spinal bulb and not in any peripheral mechanism; is an automatic and not a reflex mechanism.

(b) It opposes the view that this cause is in the activity of the expiratory centre.

(c) It gives positive proof that the agent lies rather in the activity of the inspiratory centre.

(d) It is inclined to regard the mechanism as rather a depression of vagal centre than a stimulation of the hypothetical accelerator centre.

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ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

I. The Flexion Reflex

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From the Laboratory of Physiology in the Harvard Medical School

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INTRODUCTION

Although the mammalian spinal reflexes have been studied exhaustively with the myograph method by Sherrington,[2] Graham Brown[3] and others, we have found no record of researches in which the modern quick-acting electrical recording devices have been applied to their analysis through the action currents

[1] We wish to thank Mr. McKeen Cattell for assistance in the last five experiments.

[2] Sherrington: Integrative Action of the Nervous System. 1906; Proc. Roy. Soc., vol. 81, 1909, p. 249; Journal of Physiology, vol. 40, 1910, p. 28; Quart. Journ. Exp. Physiol., vol. 6, 1913, p. 252; Journal of Physiology, vol. 47, 1913, p. 196.

[3] Graham Brown: Quart. Journ. Exp. Physiol., vol. 7, 1914, pp. 197–418, etc.

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of the motor nerves. The action currents of human muscles in voluntary contraction and on electrical stimulation of their motor nerves have been led off through the skin and recorded with the string galvanometer by Piper[4] and by Garten.[5] The same method has been used by Snyder[6] and by Hoffmann[7] in the study of the knee jerk and ankle jerk, whose reflex nature seems to be accepted by a majority of investigators. Jolly[8] has applied the same method to the study of reflex time in the case of rabbit, cat and man. Piper[9] has also examined the action currents of muscles in the turtle under reflex stimulation. Reflexes in the frog have been examined through the action currents in the muscles by Buchanan[10] with the capillary electrometer and by Beritoff[11] with the string galvanometer.

The action currents of the extensor muscles in the decerebrate cat have been examined by Buytendyk[12] with the string galvanometer, and an attempt is mentioned by him to observe the action current of the sciatic nerve under reflex stimulation. Foa[13] reported a successful observation of this sort at Vienna in 1910, but no details are given as to the animal used, the method, or the results.

With the exception of the last two experiments, we find no case in which the various records obtained from muscles have been compared with any record obtained directly from motor nerves under similar conditions. Gotch and Horsley,[14] in 1891, published an extensive study of the electrical disturbances in

[4] Piper: Electrophysiologie menschlicher Muskeln. Berlin, 1912; Pflügers Archiv, vol. 119, 1907, p. 301; ibid., vol. 127, 1909, p. 474, ibid., vol. 129, 1909, p. 145; Zeitschrift für Biologie, vol. 50, 1908, p. 393, p. 504.

[5] Garten: Zeitschrift für Biologie, vol. 52, 1909, p. 534.

[6] Snyder: American Journal of Physiology, vol. 26, 1910, p. 474.

[7] Hoffmann: Arch. für Physiologie, 1910, p. 223.

[8] Jolly: Quart. Journ. Exp. Physiol., vol. 4, 1911, p. 67.

[9] Piper: Arch. für Physiol., 1910, p. 207.

[10] Buchanan: Journal of Physiol., vol. 27, 1901, p. 95; Quart. Journal of Experimental Physiol., vol. 5, 1912, p. 91.

[11] Beritoff: Zeitschrift für Biologie, vol. 62, 1913, p. 125.

[12] Buytendyk: Zeitschrift für Biologie, vol. 59, 1912, p. 36.

[13] Foa: Zentralblatt für Physiologie, vol. 24, 1910, p. 792.

[14] Gotch and Horsley: Phil. Trans. London, vol. 182, 1891, p. 267.

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various parts of the mammalian nervous system with tetanic stimuli applied to various points. The apparatus available at that time was not capable of recording rapid and minute electrical changes with the accuracy now obtainable with the capillary electrometer made by Lucas's[15] method or with the string galvanometer. Furthermore, their attention was devoted to tracing the various conducting paths rather than to analyzing reflex responses with reference to their time relations. Einthoven[16] has studied the action currents of the vagus nerve under various conditions with the string galvanometer, and Dittler[17] has made a similar study of the phrenic nerve. This method, however, has apparently not been applied to the mammalian spinal reflexes which are so well known from the point of view of the myograph.

Buchanan[18] has presented evidence tending to show that the rhythm found in the action currents of muscles in voluntary contraction may be an intrinsic rhythm of muscle independent of any rhythm of impulses in the motor nerve supplying it. Dittler infers (vide supra) that the rhythm in muscle exactly corresponds with that in the motor nerve. As yet no evidence on either side seems to us wholly conclusive. Because of this it seems desirable to use the action currents of the motor nerves themselves where possible, as an index of central nervous action, especially when dealing with rapid rhythm. In view of the importance of rhythm in the activity of the motor centres, there seems to be a useful field for the employment of a method of recording which will eliminate the possibility of confusion arising from any natural periodicity of the muscle or the recording apparatus itself. Furthermore, in certain investigations, such as those dealing with the effects of drugs on the nervous system, it is especially desirable to use a method in which possible changes in the muscles cannot confuse the results. Motor nerves also

[15] Lucas: Journal of Physiology, vol. 37, 1908, Proc. Physiol. Soc., p. xxviii.

[16] Einthoven: Quart. Journ. Exp. Physiol., vol. 1, 1908, p. 243.

[17] Dittler: Pflüger's Archiv., vol. 131, 1910, p. 581. Cf. also ibid., vol. 130, 1909, p. 400.

[18] Buchanan: Journal of Physiology, vol. 27, 1901, p. 95; Quart. Journal Exp. Physiol., vol. 1, 1908, p. 211.

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present certain advantages over muscles for this sort of study in that they can be isolated for considerable lengths with but little disturbance to their physiological state and with absolute certainty that no shift of contact with electrodes can confuse the result by changing the demarcation current.

With these considerations in view, this study of the flexion reflex in the decerebrate mammal has been made. This reflex was chosen as being the simplest and most regular reflex obtainable in response to the simplest stimulus, the single induction shock. In the course of the experiments certain facts have appeared which have an interesting bearing on the problem of the spread of reflexes and the theory of graded synaptic resistance. The discussion of these facts and the problem they present will be reserved for a later paper. The present paper deals with a description of the general properties of the flexion reflex in the cat as recorded by the action current in the motor nerve, and a comparison of these records with those similarly obtained from the muscle innervated by the same nerve under the same conditions of stimulation.

METHOD

A. Electrical recording apparatus

We have used throughout these experiments an Einthoven string galvanometer furnished by the Cambridge Scientific Instrument Co. of Cambridge, England. During the greater part of the work this was provided with magnet coils connected in series and excited by the 220-volt direct current from the local power plant. In the last few experiments this was replaced by a low resistance coil in two parts connected in parallel, and excited by eight Edison storage cells (type B-6) arranged in series. This furnishes a magnetic field almost identical with that of the other coil, and has the advantages of dependable steadiness and complete insulation from other circuits. When the first coil was in use and excited from the 220-volt circuit, the iron core of the magnet was regularly put to earth. Since in the Cambridge galvanometer the upper end of the string is metallically connected with the iron core, this procedure, of

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course, involves putting the string to earth. With the low voltage coil this was not done except in special cases.

Three strings have been employed in the galvanometer in the course of the present investigation. The first of these, "String C," is of platinum and has an average diameter of 3μ and a resistance of approximately 5000Ω. This string was prepared from Wollaston wire by Dr. H. C. Hayes of the Physics Department at Harvard. It was used only in a few of the earlier experiments. The second, "String D," lent us by Dr. Hayes, was of silvered glass, made by the Cambridge Scientific Instrument Co., and had a dimaeter of 5μ and a resistance of about 1750Ω. This was used in the majority of the experiments. The third, "String E," was made by Dr. H. B. Williams of the College of Physicians and Surgeons of New York. It is of quartz silvered by the cathode spray method described by him.[19] It has a diameter of 1.5μ and a resistance of 16,100Ω.

With strings C and D the tension was in general adjusted at about the limit of periodicity. That is, when a resistance of the average magnitude found in the physiological circuit is connected in series with the string and the closure of a constant current recorded, the calibration curve thus obtained shows only a small over-shoot amounting to one or two per cent of the total excursion (e.g., figs. 5, 6 and 11). The tension at which this type of curve was obtained was about 62 per cent greater in the case of string D than with string C, for the inertia of the latter was greater and the air damping less. The time required for the string to reach the full magnitude of its excursion at a given tension varies according to the resistance in circuit on account of the electromagnetic damping. The resistance of the nerve under observation as included in the circuit usually lay between 15,000 and 40,000 ohms. With such resistance in circuit, string C would reach its final amplitude of excursion in about 15σ at the tension commonly used, while string D at the higher tension at which it was usually employed reached its full excursion in about 13 or 14σ. The magnification of the string which was constantly

[19] Williams: Physical Review, vol. 2, series 2, 1913, p. 402.

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employed in these experiments was 580 diameters. With this magnification string C at the tension commonly employed gave an apparent excursion of 1 cm. with a current of 8×10^{-8} amp. The tension commonly employed with string D was such that 1 cm. measured 13×10^{-8} amp.

String E on account of its extreme lightness possessed a wider range of tensions which could be used without rendering it periodic. With a tension about two and one-half times as great as that used with string D and nearly four times as great as that used with string C and with resistances of 20,000 to 40,000 ohms in series, the overshoot was only of the same order as that commonly found in the calibration curves with the other strings, i.e., about 1.5 per cent. Moreover, at this tension the full magnitude of excursion on the make of a constant current was reached in about 4σ. At this tension, therefore, it was possible to obtain far more accurate records as regards time relations than was possible with either of the other strings. Where this was desired the tension mentioned (1 cm. $= 31 \times 10^{-8}$ amp.) was used. On the other hand, excursions in response to nerve action currents are small at this tension, and since even at slacker tensions the initial portion of the excursion is quicker than is obtainable with the heavier strings, a tension equal to or less than that used with string D was employed where it was desired to compare magnitudes of disturbance.

The galvanometer was mounted on a wooden stand built solidly against the outside stone wall of the building. This position was found to be remarkably free from vibration. The wires in the circuit which included the string, were all insulated and lead-sheathed, and the lead sheaths were grounded. All switches and resistances in circuit with the string were mounted on hard rubber blocks or porcelain bases to insulate them from the tables on which they stood. Figure 1 shows diagrammatically the arrangement of wires, etc., in connection with the string. From one terminal of the string (G) a wire is led to one end of a slide wire 1 metre long having 4.8 ohms resistance. This slide wire is connected in series with resistance box (R_1) and with an Edison cell through a pole-changing switch. The other ter-

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minal of the string is led to one pole of a two-way, double-throw switch (*DS*) which connects the string either with the physiological preparation or with a substitution resistance (R_2) as desired. From the other pole of this switch a wire is led to the sliding contact on the slide wire. With this arrangement the resistance in R_1 is so adjusted that the fall of potential along the slide wire shall be 0.001 volt to 1 cm. or to 10 cm. or to any other convenient value. This makes it possible to introduce quickly into the circuit in series with the string and either the nerve or the substitution resistance any desired E.M.F. from 0.0001 volt or less to 0.1 volt in either direction; and the voltages so used are read directly from the meter scale without computation.

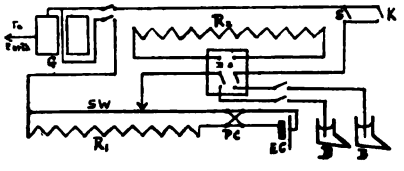


Fig. 1. Electrical connections. *G*, string galvanometer. *DS*, two way double-throw switch. *EC*, Edison storage cell. *PC*, pole-changing switch. *SW*, slide wire. R_1, resistance box to regulate compensating current. R_2, substitution resistance. *K*, spring contact key. *S*, knife blade switch. *B*,*B*, non-polarisable boot electrodes.

A simple spring contact key (*K*) is operated by hand for making calibration curves, while for permanent closure of the circuit a knife-blade switch (*S*) is arranged in parallel at the same point in the circuit. The make calibration curve is identical wherever the circuit is closed, since the excursion is always opposed by electro-magnetic damping. In Einthoven's constant current records the key is so placed that the return of the string following the break is also electro-magnetically damped. With our arrangement this is eliminated and the return is opposed only by air damping.[20] This method is sometimes useful when it is desired to show what part electro-magnetic damping plays in the record.

[20] Cf. Samojloff: Pflüger's Archiv., vol. 149, 1913, p. 492.

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B. Optical system

In dealing with complex physiological preparations it has been deemed best to simplifiy as far as possible procedures incidental to recording, so that our attention should not be diverted from the physiological part of the experiment by the need of making adjustments. To this end a Nernst lamp has been used for illumination in place of the arc lamp which is generally employed in projection work. This glows indefinitely with absolute steadiness and without need of adjustment when once placed in position. Its intrinsic brilliancy is so much less than that of an arc lamp that it would not suffice for making photographic records at the required speed and magnification without far greater economy of light rays than is found in most projection systems. This has been achieved by introducing two cylindrical lenses

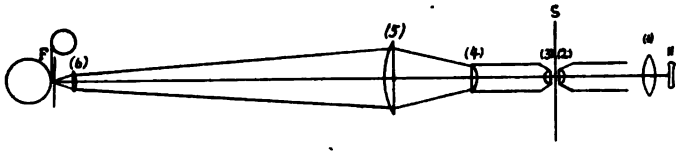


Fig. 2. Diagram of optical system. *N*, Nernst glower. (*1*), double convex lens. (*2*), condensing objective. *S*, string of galvanometer. (*3*), projecting objective. (*4*), ocular. (*5*), large plano-convex cylindrical lens. (*6*), small plano-convex cylindrical lens in camera. *F*, photographic film.

into the system. The general arrangement of lenses is indicated in figure 2. The Nernst filament is placed in a vertical position about 30 cm. away from the string. Close to the light is placed a 16 D double convex lens (1), and in the rear draw-tube of the galvanometer a 16 mm. objective (2) which serves as a condenser. These lenses and the light are so placed that an image of the Nernst filament is focused in the plane of the string and again at the plane of the recording surface, and their positions are further selected so that this latter image of the Nernst filament shall have the same width as the film. In this way the maximum available illumination is secured. In the front draw-tube are placed a 16 mm. Zeiss objective (3) and a Zeiss No. 8 compensating ocular (4) for projecting the image of the string. About 20 cm. in front

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of the ocular, and mounted on a separate stand, is a large 6 D plano-convex cylindrical lens (5). In the receiving camera is a plano-convex cylindrical lens (6) of 4.9 cm. principal focal distance corresponding to that in other recording apparatus employed with the string galvanometer. Behind this lens is the recording film which travels in a vertical direction. The distance from the string to the film is 133 cm. Without the large cylindrical lens (5) the small one would only receive a small portion of the light emerging from the ocular, but with this lens properly placed practically all of the light is concentrated in a narrow horizontal beam falling on the smaller lens; this is, in turn, concentrated so that nearly all of it falls on the narrow slit in front of the film. With this double system it is impossible to obtain a sharp horizontal line of light from the smaller cylindrical lens as is commonly done in other recording apparatus, and it is necessary to rely for definition on a narrow slit in front of and as close as possible to the film. In our experiments the slit has been such that a band of light about 0.8 mm. wide falls on the film at a given instant. For very minute analyses involving exact time measurements this would not be satisfactory, but for the purposes of this research where time measurements more accurate than to 0.0005 second were not required the method has been efficient and especially convenient. The placing of these cylindrical lenses has been done empirically by shifting back and forth until the maximum illumination is found. In obtaining a sharp focus of the string it is essential that the axis of curvature of the big cylindrical lens (5) should be exactly parallel with the string (i.e., vertical), and to insure this a fine adjustment with rack and pinion is provided whereby this lens is rotated in its own plane about the optical axis till the best definition is obtained.

In the last four or five experiments an arc lamp was used for illumination. This made it possible to dispense with the large cylindrical lens and to make the light fall in a sharp line across the film. This rendered measurements of time more accurate than with the system just described.

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C. Photographic recording apparatus

The recording camera was built around a Sandstrom electric kymograph; it was especially designed to record large numbers of observations in rapid succession and to work in daylight. For this purpose it has proved eminently satisfactory. The Sandstrom kymograph is driven by the power plant current and is provided with a governor which makes possible an approximately constant speed. It is also provided with an extensive series of gears by which at constant velocity of the motor a wide range of velocities of the drum is obtained.

The film is reeled off from a spool, around the kymograph drum and on to another spool in a separate receiving chamber. The velocity of the film is determined wholly by the kymograph drum against whose surface the film is pressed firmly by rubber rollers held in place by springs. The spool in the receiving chamber is turned by a spring clock-work device. The pull of this is adjusted merely to take up the slack of the film and is not made strong enough to modify the velocity imparted to it by the kymograph drum. The arrangement of these parts is shown in vertical section in figure 3.

The film used is 3½-inch moving picture film supplied by the Eastman Kodak Co. in 50-foot rolls. It comes on simple wooden spools with holes drilled in the ends. In loading the camera the spool bearing the film is set up at *F* (fig. 3) in a frame in which its unwinding is opposed by a slight friction. From here it is led around the surface of the kymograph drum and out through an opening (*O*) by which it connects with the receiving chamber (*R*).

The kymograph is provided with a drum having a circumference of exactly 50 cm. In order to economize film and to render the camera compact a smaller brass drum was substituted for this. It has a circumference of 14.1 cm. and has flanges at the ends to keep the film from lateral excursion. It was found necessary to supplement these with guide plates between the feeding spool and the drum making it impossible for the film to ride up on the flanges and get jammed.

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The drum is so placed that the film passes as close as possible behind the slit (*S*), and then with the least possible distance to traverse into the receiving chamber. The purpose of this chamber was to make it possible to remove for development the exposed portion of the film without having to dismount the whole camera and take it to the dark room. It was made, according to a plan suggested by Dr. E. S. Kilgore, of two concentric brass cylinders, one fitting close inside the other. The ends are closed by plates, each of which is fastened rigidly to one of the cylinders and has a hole in its centre for the shaft of the receiving spool inside to pass through. Large flanges at the ends of the receiving spool prevent any light which enters these holes from reach-

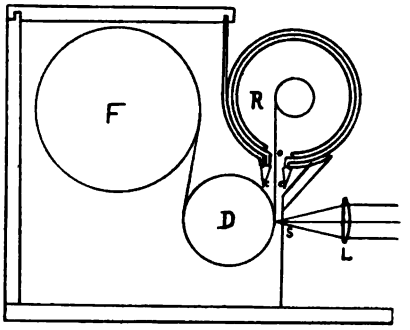


Fig. 3. Vertical section through recording camera. *F*, spool of film. *D*, kymograph drum. *O*, opening from camera to receiving chamber. *R*, receiving chamber. *C,C*, spring clips which close opening and grip the film when receiving chamber is removed. *S*, adjustable horizontal slit. *L*, cylindrical lens.

ing the film. A lengthwise section through the chamber with the spool in place is shown in figure 4.

To admit the film a slit is cut lengthwise in each cylinder, and when the chamber is fitted to receive the film these are set to coincide with each other and with the opening (*O*, fig. 3) in the camera. Two lips fastened to the outer clyinder press open a pair of spring clips (*c*, *c*) at the opening, which when the receiving chamber is removed close on the protruding end of the film and prevent light from entering the camera. Before removal, the inner cylinder is rotated within the outer so that the slits no longer coincide and the film is held firmly as it enters between the overlapping portions of the cylinders. The chamber is then raised and the film cut between it and the camera. When thus closed, the receiving chamber has proved absolutely light tight.

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When the receiving chamber is in place the shaft of the spool is connected by a disc coupling with the shaft of the spring device whereby slack is taken up and the film wound in as it leaves the drum.

The slit through which the light enters in front of the drum can be varied in width by fine adjustment screws. In front of the slit is a shutter which may be closed by hand when the apparatus is not in use. This is not found necessary as a rule for on account of the shape of the camera only about an inch of film can be fogged with the shutter left open. The position of the small cylindrical lens which is mounted in the camera in front of the slit is regulated by fine adjustment screws to give the best concentration of light.

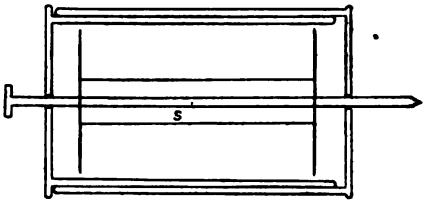


Fig. 4. Longitudinal section of receiving chamber showing receiving spool (*S*) within.

The kymograph is operated by setting the electric motor in motion, and when this has attained its full velocity, by turning a lever which throws in the gears for the desired speed of the drum. When this is done, the drum attains its full velocity practically instantly. Several speeds of the drum are available, but only the two highest have been used in these experiments. These two speeds are intended with the standard drum to give surface velocities of 50 cm. and 100 cm. per second. With the small drum used in the camera the velocities are, of course, reduced in proportion to the circumference and are 14 cm. and 28 cm. respectively.

With the illumination already described it was found that even at the highest speed fair contrast was obtained in the exposed film with ordinary developer. By using a concentrated developer warmed to about 28°C. excellent contrast was obtained without injury to the film.

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It was found that if the metal handle by which the gears are shifted was grasped with the bare hand, oscillations were induced in the string synchronous with the revolutions of the armature in the motor. To obviate this difficulty a hard rubber handle was fitted over the regular handle and to this was fastened a brass cross bar. To the latter was soldered a copper wire by which it was connected to earth. As an additional precaution, a tin shield, also led to earth, was fitted over the whole motor. When the motor is operated by the grounded brass cross bar, no oscillations of the string are induced.

Time is recorded on the film by means of a tuning fork kept vibrating by an electro-magnet with platinum contact interrupter. One limb of the tuning fork, which makes 100 complete vibrations per second, is placed in the path of the beam of light.

For the purpose at hand this apparatus has proved quite satisfactory. Its salient feature is the facility with which large numbers of observations can be made in quick succession and with great economy of film. With a little practice it has been found possible to throw in the gears, stimulate the nerve with the other hand and throw out the gears in the space of 0.16 second, as is shown by the time-marker on the exposed film. Longer exposures can be made at will, up to the limit of the film, where longer series of events are to be recorded. No readjustment of any part of the recording apparatus is required between exposures. It is possible, therefore, with a 50-foot film, to take at the highest speed of the drum over 250 successive exposures as rapidly as is desired, or double that number at the second speed.

D. Stimulation apparatus

For stimulation a Berne inductorium[21] was used, except in the first experiment (preparation 2) from which records have been reproduced; in this experiment a Gaiffe coil was employed. The Berne coil was calibrated with Martin's apparatus in accordance with his method.[22] The primary current was supplied by an

[21] This coil was exactly like that shown in figure 9 of Martin's book. Measurement of Induction Shocks. New York, 1912, p. 30.

[22] Martin: Loc. cit., p. 55.

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Edison storage cell and determined by an ammeter in the circuit. To show the instant of stimulation there was introduced into the primary circuit a small signal magnet whose recording lever is very light and when released from the magnet on the break is pulled back with great rapidity by a wire spring. A narrow strip of paper cemented to this lever was placed in the path of the beam of light before the slit in the camera. The quickness of this signal was tested by connecting it in parallel with the string galvanometer, diverting just enough current through the latter to give a good excursion. On examining the record of the break of a constant current with this method at the highest speed of the film the lag was found to be extremely small. Experiments with more recently constructed apparatus capable of determining time to about 0.1σ have shown that the first perceptible excursion of the signal magnet occurred between 0.6 and 0.7σ after the current was broken. In all time measurements the nearest black line in the film, showing where it had been at rest with the shutter open, was used as a base. Since it was necessary to lead the wires supplying the signal magnet close to the motor which operates the drum, these were enclosed in lead sheathing and the lead put to earth, to insure further against the induction of electrical disturbance, in the string.

In approximately the first half of the experiments the Martin knife-blade mercury key[23] was employed. This proved satisfactory at first, but through a defect which later appeared in the operation of the key at our disposal it was found to make a brief secondary closure of the circuit after breaking, thus giving rise to three successive shocks. Sometimes this was shown by the signal magnet, but more often the secondary closure was of too brief duration to deform the signal record, yet it was evidenced in the response of a nerve under direct stimulation which yielded two or three separate action currents instead of one.[24] On this account it was replaced by a key made on a plan recommended by Dr. H. B. Williams. This consists of a

[23] Martin: Loc. cit., p. 63.

[24] This defect was afterwards remedied by the introduction of a strip of steel to guide the knife-blade. See Martin: This journal, vol. 36, 1915, p. 237.

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small glass cup containing clean redistilled mercury connected with one terminal, and a rod of copper tapered to a sharp point and amalgamated, connected with the other. This rod is dipped into the mercury and withdrawn by means of a hard rubber lever operated by hand. This key can be relied upon to give clean makes and breaks. There may be slight variations in the intensity of the break shocks dependent on the speed with which the copper point is withdrawn from the mercury; but in a control experiment with threshold stimulation of a frog's muscle the strength of the shocks was found not to vary by as much as 2 per cent when the speed of withdrawal was purposely varied as widely as it well could be in operation by hand. This means that for the degree of accuracy requisite in these experiments it was quite satisfactory.

In nearly all of the experiments the primary circuit included in series the cell, the key, the signal magnet, the primary coil, the ammeter and a small resistance coil (2, 3 or 4 ohms) to reduce the current to the desired value. The strengths of the stimuli were determined in Z units according to Martin's method.[25] In three experiments in which weak stimuli were desired the circuit was divided, the greater part of the current going through the signal magnet which would not operate on less than 0.20 amp., while only about 0.1 amp. was allowed to go through the primary coil. The provision of a shunt rendering the break less abrupt through inductance, caused the break shocks to be far less intense than would be the case on complete interruption of a current of the same strength. In consequence, it was impossible to apply the Martin method to the evaluation of these shocks, and it was quite evident from a comparison by the two methods of thresholds in the same preparation that the shunt greatly reduced the physiological intensity of the shocks.

E. Experimental procedure

Throughout this investigation decerebrate cats were used exclusively. Decerebration was performed under ether anaesthe-

[25] Martin: Measurement of Induction Shocks, p. 73.

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sia in the manner described by Sherrington and one of us.[26] The essential features of the operation are the ligation of the carotids and the removal of the entire brain above the level of the posterior corpora quadrigemina.

Although etherization was stopped simultaneously with the completion of decerebration the flexion reflex was usually not obtainable for an hour or more and was seldom vigorous until two or three hours had elapsed. In order that the observations should be made after the vigor of the reflex had been fully regained, the animal was usually left undisturbed for an hour or two after decerebration before beginning the subsequent preparations which required from one and one-half to two and one-half hours. From the beginning of anaesthetization the animal was kept on an electric heating pad whereby the body temperature was kept approximately normal until just before the observations were begun. The pad was then disconnected to avoid the danger of leakage of current.

The motor nerve selected was the peroneal. The principal muscle which it innervates is the ankle flexor, tibialis anticus; it is readily dissected out over a distance of 12 cm., in which it gives off no branches, between the hip and the knee; this renders it a favorable nerve to work with. We desired if possible to record the action currents in the nerve without disturbing its continuity or paralysing the muscle. To place two electrodes on the uninjured nerve and thus record diphasic action currents while the muscle was still innervated was not found practicable for the following reasons: First, it was almost impossible to maintain contacts between electrodes and nerve which could be relied on not to shift, without making contact with other tissues, or else putting a strain on the nerve which would rapidly impair its conductivity. Furthermore, it was difficult, even with the maximum length of nerve available, to separate the electrodes far enough to enable the string to follow the phases of the action current, overlapping as they do on account of the high velocity of the nerve impulse unless several centimeters

[26] Forbes and Sherrington: This journal, vol. 35, 1914, p. 367.

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intervene between the leads. Finally, it is only in case the impulses are sent down the nerve trunk 'in a volley,' i.e., simultaneously in all the fibres, that we should expect to obtain an intelligible record with the diphasic method, since otherwise the second phase in some fibres would neutralize the first phase in others and a confused record at best would be obtained. Since it is not at all certain that central discharges travel simultaneously in all the fibres of a given nerve trunk,[27] it seemed best to simplify the records by rendering the action currents monophasic.

An attempt to do this without injuring the nerve was made in the following way: One electrode was placed on the motor nerve, and the other (indifferent) electrode on a muscle (paralyzed by section of its nerve) on the other side of the leg. It was found, however, that with this arrangement, the action current of the innervated muscle following that of the nerve introduced itself into the circuit and confused the result. The method was therefore abandoned.

The method finally adopted was to cut the nerve at its entrance to the muscle and render the part in contact with the distal electrode inactive. In one experiment this was done in the classical manner by devitalizing the distal portion in hot Ringer solution. In all other experiments, however, the nerve was simply crushed with a hemostat to block the impulses at a point about midway between the leads. This method saves time, and no appreciable difference in the result could be detected, except that there is less demarcation current to compensate.

The operative procedure subsequent to decerebration was as follows: A long incision was made in the lateral aspect of the thigh, from hip to knee. The biceps femoris muscle was dissected away from the fascia along its anterior margin and its insertion divided; it was then reflected back, thus exposing the sciatic nerve and its terminal branches as far as the knee, and opening a region of easy access to the femur for the insertion of a clamp. The muscles covering the sciatic nerve in the neigh-

[27] Buytendyk: Loc. cit., p. 44.

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borhood of the hip were then divided and the nerve laid bare almost to its point of emergence through the great sciatic notch. The peroneal nerve was then dissected carefully from the bifurcation of the sciatic to its entrance to the tibialis anticus muscle. It was also dissected away from the rest of the sciatic trunk as far back as the hip. With fine scissors this operation can be performed rapidly and with little or no trauma to the nerve. The popliteal nerve was then ligated near the knee and cut distal to the ligature. Platinum stimulating electrodes arranged to give ascending break shocks were then temporarily applied to the central end of the cut popliteal nerve, and the threshold for reflex contraction of the tibialis anticus was determined in Z units for break shocks. When only the activity of the motor nerve was to be studied the peroneal nerve was then crushed at a point about 3 cm. from its entrance to the muscle and cut 2 or 3 cm. distal to the crush. To the popliteal nerve was then applied a pair of Sherrington shielded electrodes for afferent stimulation. A clamp was fastened to the femur and secured in a rigid stand, thereby practically immobilizing the limb. Sometimes, further to insure immobility, the hamstring nerve was cut; sometimes it was left so that contraction in the muscles should serve as a visible index of the reflex. The stimulating electrodes were now connected with the secondary coil for ascending break shocks and the peroneal nerve led through a small opening into a moist receiving chamber where it was laid across a pair of non-polarizable "boot" electrodes about 3 cm. apart with the crush approximately midway between. This receiving chamber is a small hard rubber box clamped firmly to a stand by which it is held close to the animal's hip. The boot electrodes are fitted in notches in the wall of the chamber where they are held in place with wax. The hole through which the nerve enters the chamber was plugged with a paste of kaolin and Ringer solution which served both to conserve the moisture within the chamber and to steady the nerve and prevent any vibration imparted to it by motion of the animal from being transmitted to the electrode contacts.[28] As a further

[28] Cf. Dittler: Loc. cit., p. 583.

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safeguard, the nerve was drawn into the chamber far enough to allow a little slack between the kaolin plug and the proximal electrode. Only a short length of nerve was exposed to the open air between the animal's body and the entrance to the moist chamber. In most experiments this was protected from drying by a flap of skin cut from the thigh and wrapped around it.[29] As soon as the nerve had been placed on the electrodes the circuit through the string was closed, and the demarcation current compensated by means of the cell and slide wire described above. Except when effects of fatigue were especially sought a rest of at least ten seconds was allowed between successive observations on the reflex.

In nearly all experiments, after a series of responses in the nerve had been recorded, it was severed at the hip and stimulating electrodes applied at the central end of the isolated portion, arranged for descending break shocks. A series of responses was then recorded to afford a comparison of the responses in the nerve under reflex and under direct stimulation.

EXPERIMENTAL RESULTS

A. The typical flexion reflex

The flexion reflex has been examined by the method described above in twenty-five decerebrate cats. The monophasic electrical responses obtained from the peroneal nerve in the flexion reflex induced by single break shocks applied to the popliteal nerve are, in the majority of cases, such as are shown in figures 5 and 6. In figure 6, (A) in all cases shows the reflex responses to maximal or approximately maximal break shocks, (B) shows the responses to maximal break shocks applied directly to the motor nerve, (C) shows the calibration curves obtained with

[29] In our first experiments before the moist receiving chamber was devised, we used a method for which we are indebted to Dr. H. B. Williams, which consisted merely of leading the nerve over a glass hook and then, with enough slack between to insure steady contacts, over the boot electrodes set up in the open air. This sufficed for a brief experiment, but the drying of the nerve limited the time available for satisfactory observations far more than when the moist chamber was employed.

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the same tension of the string and the same (or approximately the same) resistance in circuit as was included when the nerve was under observation. This resistance in each case was de-

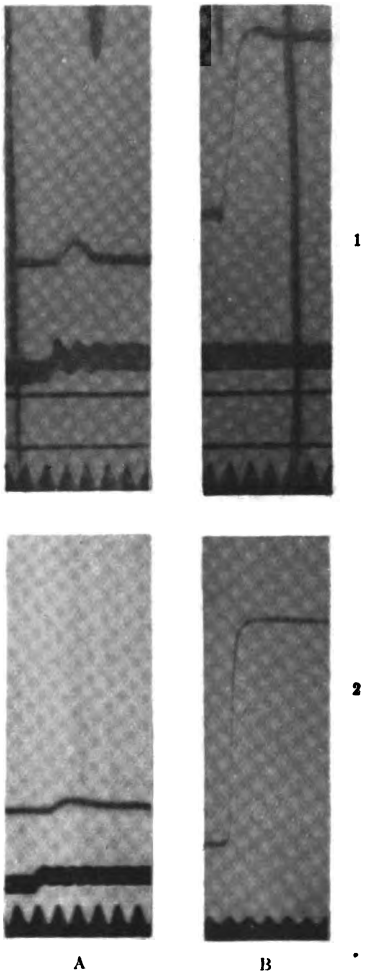


Fig. 5. *A*, typical monoplasic reflex responses of peroneal nerve to single maximal induction shocks. *B*, calibration curves, see text. *1*, string *C*; *A*, preparation 2; *B*, $\frac{20 \text{ millivolts}}{\text{string} + 74{,}000 \text{ ohms}}$. *2*, string *E*; *A*, preparation 20; *B*, $\frac{20 \text{ millivolts}}{\text{string} + 28{,}000 \text{ ohms}}$. In this and all subsequent records the top line shows the excursions of the string. The second line (where present) shows the time of stimulation. A fall in this line shows the make, a rise the break of the primary current. The small oscillations following the break are vibratory and do not indicate secondary closure of the circuit. The bottom line records time; each complete vibration = 0.01 second.

termined in the following manner. The demarcation current was compensated and then the compensating voltage was shut off from the circuit which otherwise remained unchanged. Thus was determined the excursion of the string in response to the

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uncompensated demarcation current. The two-way double-throw switch (*D.S.*, fig. 1) was then thrown to the substitution resistance box (R_2) and a resistance found through which the compensating voltage produced the same excursion.

B. Reflex time

The first property of the responses to consider is the latency or reflex time. This interval between the stimulus and the response was measured on the film in over two hundred records obtained from seventeen preparations.

Fig. 6. Description in text. Strength of induction shocks in *Z* units, and values of calibration curves as follows:

1. Preparation 5. String C.
 Stimulus, A, $\frac{584}{K}$ Z; B, $\frac{39}{K}$ Z; C, $\frac{10}{\text{String} + 46{,}000}$
2. Preparation 6. String C.
 Stimulus, A, $\frac{104}{K}$ Z; B, $\frac{26}{K}$ Z; C, $\frac{10}{\text{String} + 40{,}000}$
3. Preparation 8. String D.
 Stimulus, A, 73 Z; B, 73 Z; C, $\frac{10}{\text{String} + 40{,}000}$
4. Preparation 9. String D.
 Stimulus, A, 38 Z; B, 38 Z; C, $\frac{10}{\text{String} + 37{,}000}$
5. Preparation 12. String D.
 Stimulus, A, 197 Z; B, 197 Z; C, $\frac{20}{\text{String} + 39{,}000}$
6. Preparation 13. String D.
 Stimulus, A, 59 Z; B, 6.8 Z; C, $\frac{20}{\text{String} + 20{,}000}$
7. Preparation 19. String E.
 Stimulus, A, 75 Z; B, 17 Z; C, $\frac{50}{\text{String} + 28{,}000}$

In Nos. 1 and 2, and in Fig. 11 No. 3, the *Z* units are given divided by an unknown constant *K*. This signifies the introduction of a shunt in the primary circuit which reduces the intensity of the break shocks (see p. 132). In the fractions indicating the currents used in calibration curves the numerators denote millivolts, the denominators ohms in series with the string. The tension of the string in No. 7 is higher than in any of the others. In No. 6C, the magnetic field was about half as strong as in all other records.

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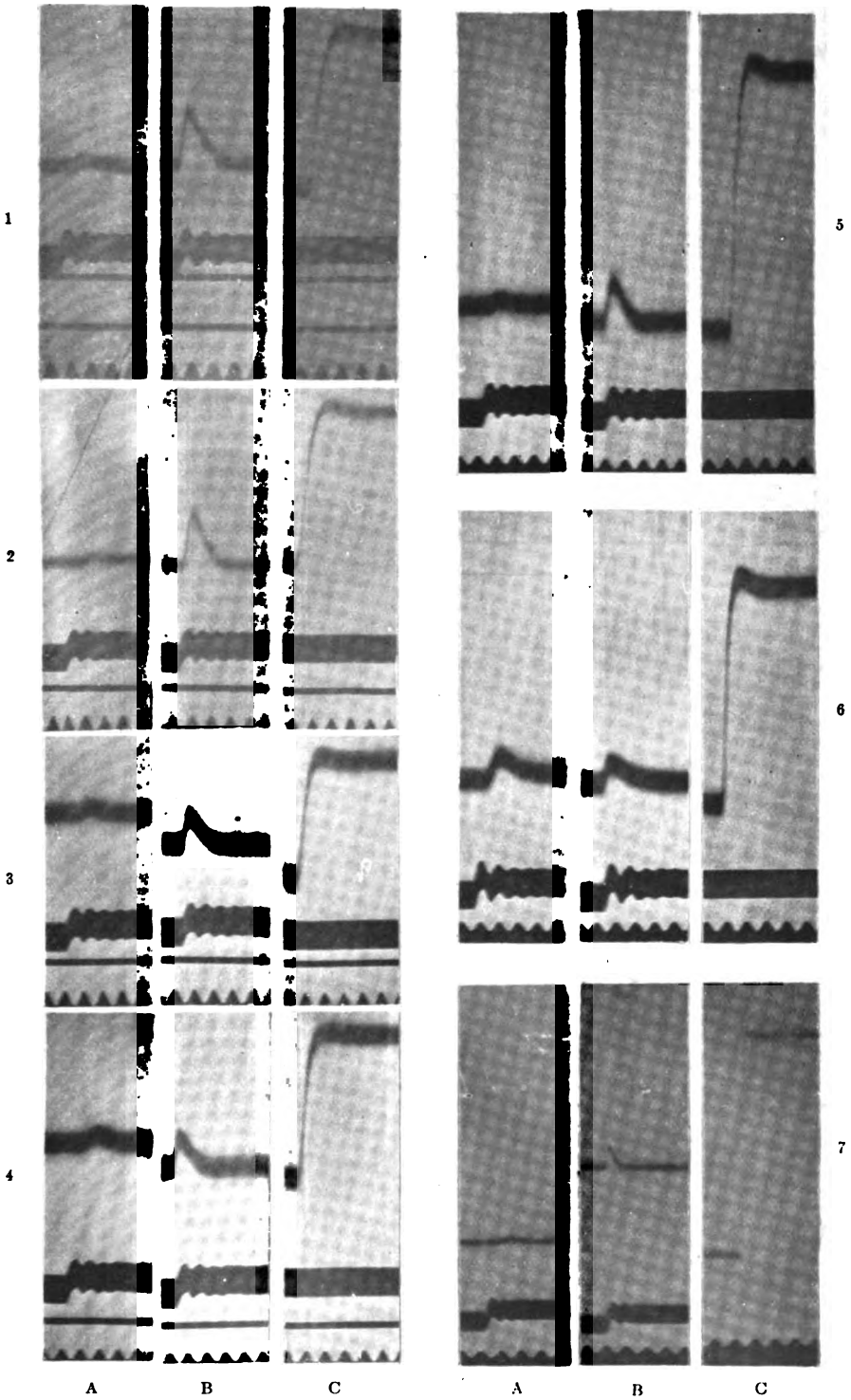


Fig. 6

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As has been indicated above, time measurements can be made with an error of less than 0.5σ provided the excursion of the string has been sufficiently abrupt. In the majority of reflex responses this condition has not been realized, and the point of departure of the string from its resting position has been so ill defined that an error of nearly 1σ may exist. For example, in one record of average clarity the measurement made between the first motion of the signal magnet and the first motion of the string indicated a time interval of 9.5σ, and it could only be stated with certainty that the time lay between 8.7σ and 10.2σ.

It has been noted above that the signal magnet had a delay of about 0.6σ. Consequently, this amount was in each case added to the time as measured by comparison with the signal magnet curve. In some experiments, for reasons which will be discussed in a subsequent paper, an electrical disturbance passing through the string at the instant of stimulation was large enough to show a small notch in the record (fig. 9). Where this was clearly marked, time measurements were taken directly from it instead of from the signal magnet. Where they were taken from both in the same records, they showed an average difference agreeing fairly well with the lag of the signal magnet as measured with the more accurate apparatus. Some of the records seem to show that the delay of the signal magnet varied somewhat, but in all cases it seems to have been so near its average value, 0.6σ, as not to introduce an additional error of more than 0.3σ or 0.4σ at most.

The results of these measurements are as follows. The smallest individual measurement of reflex time was 7.7σ; the largest was 12.8σ. An average of all the measurements for each preparation was taken, and the smallest average was 8.1σ, the largest 11.6σ. A general average made up of the averages for the separate preparations was 9.6σ. One experiment was especially favorable for accurate time measurements. The arc lamp was employed for illumination and the preliminary notch in the record showing the instant of stimulation was very sharp and well defined. The latencies measured in three records from this preparation were 8.0σ and 8.3σ and 8.0σ, making an average

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of 8.1σ. These measurements can be relied on to be within 0.4σ at most of the true value. The record showing a latency of 8.3σ is reproduced in figure 9.

In interpreting these results the following variables must be considered: the lengths of the afferent and efferent peripheral nerves lying in the reflex path, the body temperature and the temperature and extent of the exposed portions of the nerves. In all cases the stimulating electrodes were applied to the popliteal nerve at a point between 3 cm. and 6 cm. from the hip; in a great majority this distance lay between 4 cm. and 5 cm. In all but one experiment, the proximal leading off electrode touched the peroneal nerve between 7.5 cm. and 9.5 cm. from the hip, usually between 8 and 9 cm. A dissection of the lumbosacral plexus in an average sized cat showed that in the case of the nearest roots involved in the reflex the afferent fibres travel 8.5 cm. from the hip before entering the cord, and 9.4 cm. in the case of the farthest roots. The corresponding distances for efferent fibres are 8.5 cm. and 9.2 cm. Since we are concerned with the earliest response, it is probable that the shortest path is the one to consider. In all experiments the peripheral nerves were dissected out from the surrounding tissues from the hip to the points of stimulation and leading off. The afferent nerve after application of the shielded electrodes was covered by the ham-string muscles whose temperature probably remained somewhat below that of the rest of the body throughout the experiment. The portion of the motor nerve distal to the hip was exposed to room temperature, about 21°C. That portion of the sciatic nerve central to the hip was at body temperature. This was not recorded during the observations, but in almost all cases it was maintained between 35°C. and 38°C. until just before the commencement of the observations. Two notable exceptions were associated with exceptionally long reflex times. One of these, yielding the longest average reflex time of all (11.6σ) was accidentally allowed to cool through a defect in the heating pad. At the beginning of the observations the body temperature was found to be 32.7°C.; about an hour after the end of the experiment it was 29.3°C. The next longest average time (11.2σ)

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occurred in an animal whose temperature, though not taken immediately before the experiment, may yet be inferred from earlier and subsequent observations to have been between 32°C. and 33°C. during the experiment. The shortest average time (8.1σ) occurred in an animal whose temperature remained at about 36°C. throughout. From these facts it appears that the longest times recorded are the result of cooling of the animal to an abnormal degree. An average of the remaining preparations after the exclusion of those in which excessive cooling probably rendered the delay abnormal, gives the observed reflex time as 9.1σ which is probably more nearly normal than the higher value given above. Still it is not improbable that if body temperature had been maintained strictly normal in all experiments, an average nearer 8σ than 9σ might have been obtained. An experiment has been quite recently made with the new apparatus already alluded to, with a film operating at 44 cm. per second. The proximal leading-off electrode was about 7 cm. from the hip, the body temperature between 34° and 35°C. A series of six records gave an average observed reflex time of 7.7σ. Later, when the body temperature had fallen to nearly 32°C., an average of 8.0σ was obtained. The figures are probably more accurate than those with the slower apparatus, but are also probably those of an unusually quick preparation. There is some question whether the variation in readings from individual preparations is due mostly to the error inherent in making accurate measurements of such small distances or whether there was in reality any considerable variation in the reflex time in a given preparation. We feel certain that in some cases at least the observed variation exceeded the range of observational error and showed a real variation between successive records. No correlation could be found between these variations and the strength of stimulus. This may not prove that no such correlation can exist with appropriate stimuli, for practically all our observations suitable for measurement were those in which maximal and supra-maximal stimuli were employed.

In estimating the "reduced reflex time," or time of transmission through the spinal cord, from the above figures it seems valid

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to assume the velocity of impulses in those portions of the nerves which were at body temperature to be 120 metres per second.[30] The velocity in the portion of the motor nerve at room temperature probably was more nearly that commonly found in amphibian nerves at room temperatures (about 30 metres per second). The best measurements at our disposal made at room temperature with the same apparatus used in these experiments show a velocity of about 24 meters per second for impulses in the nerves which we are dealing with. These afford only a rough approximation, and the true value is probably somewhat nearer the Helmholtz figures. Assuming a velocity of 30 metres per second for this part, the time to be subtracted from the total reflex time will be between 2.5σ and 3.2σ, almost always between 2.7σ and 3.0σ.

In one instance in which the proximal lead was brought 2 cm. nearer the hip between two sets of observations no shortening of reflex time appeared to result, although the increased cooling of the animal cannot have amounted to more than about 0.6°C. in the interval. We can only guess at the rate of conduction in the short length of afferent nerve covered by the dissected muscle distal to the hip, certain only that it will lie somewhere between 30 m. and 100 m. per second. The difference between these extremes will make a difference in the subtraction amounting to about 1σ. If we assume a medium rate of 60 m. per second the error will probably be insignificant. At this rate the subtraction will be between 0.6σ and 0.9σ. The total deduction for the cooled portions must lie, then, between 3.1σ and 4.1σ; on average it will be about 3.6σ. The deduction for the portions of the nerves lying proximal to the hip should have a practically constant value of approximately 1.4σ. Making the average deduction from the average observed latency the average reduced reflex time is about 4.1σ. From the figures given above, it is safe to say that the extreme limits between which the "reduced reflex time" must normally lie are 6.5σ and 2.0σ. This allows for making the maximum subtraction from the minimum

[30] Piper: Pflüger's Archiv., vol. 127, 1909, p. 474. See also Jolly: Loc. cit. and Hoffmann: Loc. cit.

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observed time and vice versa, and the latitude is probably excessive. We think it safe to conclude that in the flexion reflex in the cat at normal temperature the "reduced reflex time" lies, in general, between 3σ and 5σ. The recent experiment with the high speed film furnished more definite data than most of the others, and in this the reduced reflex time is estimated at about 3.4σ, certainly between 3.0σ and 3.8σ. The results of these measurements are in strikingly close agreement with those obtained by Jolly[31] who deduced as the "synapse" time of the flexion reflex in the spinal cat 4.3σ. The values are considerably smaller than that which Hoffmann[32] deduces from his experiments on the tendon reflex in man or that which Buchanan[33] and Wundt[34] have found in the frog. However, the diversity in size between the spinal cords of the cat and of man may well account for the former difference, and the diversity in temperature for the difference found between the cat and frog. It is important also to note that Hoffmann was dealing with a tendon reflex (proprioceptive) in extensor muscles, while we are dealing with a different type of reflex involving only flexor muscles.

C. Other properties of the reflex response

Comparison of the typical reflex response with that of the same nerve to direct stimulation (fig. 6, columns A and B) reveals two other striking differences besides the latencies. First, the magnitude of disturbance is in all cases much smaller with maximal reflex stimulation than with maximal direct stimulation. Secondly, the disturbance when it appears is far less abrupt in its onset in the reflex than in direct stimulation; that is, it requires more time to reach its greatest magnitude. No. 6 in figure 6 does not contradict the first proposition as it might appear to. The direct stimulus in this case was considerably below maximal value, the reason being that in this experiment the

[31] Jolly: Loc. cit.

[32] Hoffmann: Loc. cit. Cf. also Sherrington: Integrative Action of the Nervous System, p. 19.

[33] Buchanan: Quart. Journ. Exp. Physiol., vol. 1, 1908, p. 1.

[34] See Starling: Human Physiology, 1913, p. 342.

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defect in the circuit breaking key already referred to was at its worst and the observation shown at B was the only one obtained from this nerve in which a single impulse was recorded. However, the fact that these two excursions are the same height makes the contrast between the time relations all the more evident.

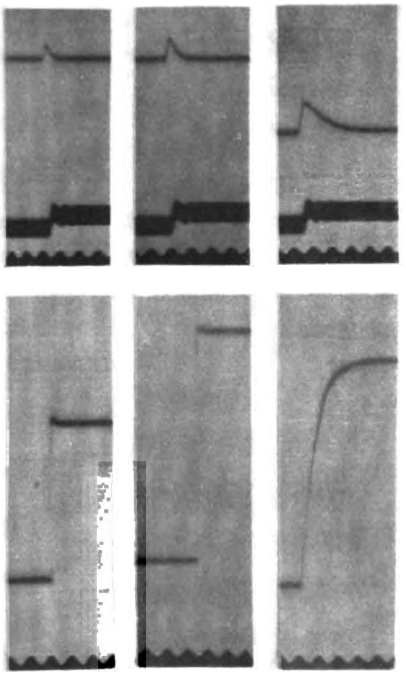
The extent of this difference in the rapidity of development of the electrical disturbance is difficult to determine exactly. No attempt has been made to apply to the curves the mathematical analysis expounded by Einthoven, but a glance at the calibration curves will show that the string cannot follow accurately the potential changes in the nerve impulse under direct stimulation. Figure 7 shows three records of maximal impulses in the same nerve stimulated directly, within a few minutes of each other at three different tensions of the string. In A the tension was such that 1 cm. excursion = 62×10^{-8} ampere, in B the string was twice as slack as in A, i.e., 1 cm. = 31×10^{-8} ampere); in C it was ten times as slack as in A (1 cm. = 6.2×10^{-8} ampere); in B the string was at about the limit of periodicity as appears from the corresponding calibration curve. At the highest tension (A) in this series the full excursion in the calibration curve is reached in 2.2σ; in B the full excursion is reached in 5.3σ; while in C it is only reached in about 30σ. In the corresponding records of action currents the durations of the ascending limbs of the curves are approximately 1.3σ, 1.9σ and 4.7σ respectively. Thus even at the highest tension the string has not had time to reach its full excursion before the maximum of the action current is over. The time of maximum potential difference probably occurs at approximately the steepest point in the curve, i.e., less than 1σ, possibly only 0.5σ, after the beginning of the disturbance.[35]

In the case of the reflex response the string must far more nearly follow the electrical disturbance relatively than in the case of direct stimulation. But even here the lag is probably such as to render appreciable the difference between the curve

[35] Cf. Erlanger and Garrey: This journal, vol. 35, 1914, p. 398, footnote. See also Gotch: Journal of Physiology, vol. 28, 1902, p. 405.

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traced by the string and a true curve of potential difference plotted against time. Yet the rise in the curve from the base line is so gradual that we may safely assume that its distortion by the lag of the string is insignificant in the case of the reflex



A B C

Fig. 7. Description in text. Preparation 19. String E.

A, Stimulus, 75 Z; Calibration $\frac{80}{\text{String}} + 40{,}000$

B, Stimulus, 75 Z; Calibration $\frac{50}{\text{String}} + 28{,}000$

C, Stimulus, 75 Z; Calibration $\frac{10}{\text{String}} + 28{,}000$

compared with that in the case of direct stimulation. Since the lag of the string enters equally into the records of both events but proportionally retards the phases of the direct response far more than those of the reflex response, it follows that the real difference in the abruptness of development of the

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electrical disturbance is even greater than appears in the records. Considerable variation appears in the shape of the reflex records as obtained from different preparations; in general, the rise from base line to maximum occupies a time varying from 4σ to 9σ, the lower figure being obtained only with a fairly tight string. From a study of the calibration curves and records of direct stimulation at various tensions of the string, it may be inferred that the above figures indicate a time varying roughly from 3σ to 6σ occupied by the reflex electrical disturbance in its increase from zero to its maximum. It may be noted that the time occupied in the subsidence of the process is, as nearly as can be judged, about the same in both the reflex and the direct response.

In general, it may be said that as compared with the direct response, the reflex action current appears after a latency of about 9σ, then rises to a maximum which is reached from four to ten times as long after its onset as is the case in the impulsed directly evoked from the nerve by a single shock; and the maximum when reached is much smaller even in a maximal reflex than is evoked by maximal stimulation of the nerve.

In connection with the magnitude of disturbance the observation of Camis[36] is important. He reported that maximal stimulation of either the peroneal or popliteal nerve alone failed to evoke as much reflex contraction of the flexor muscles as maximal stimulation of both together. In other words, neither of these nerve trunks was able alone to evoke the maximum response of which the reflex centre was capable. In view of this, we should not expect to find the response in the motor nerve in the reflex as great as when all its fibres are excited electrically. Furthermore, it must be remembered that nearly half of the fibres in the peroneal nerve are afferent and play no part in the motor discharge from the centre, and yet these fibres undoubtedly contribute a full share in the response to direct stimulation. There is some doubt whether even these two considerations taken together can explain alone the great difference in magnitude between the reflex and the direct response, considering the

[36] Camis: Journal of Physiology, vol. 39, 1909, p. 228.

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vigor of the reflex contraction which can be evoked by a single shock. Perhaps we shall have to seek further yet to account fully for the difference. The discrepancy in the time relations may furnish some clew.

For the strikingly more gradual development of the reflex response than of the direct, two conceivable explanations present themselves.

(1) It might be inferred that impulses arising in a reflex centre are different in kind from those evoked by an artificial stimulus directly applied to the nerve trunk. In one case the nerve fibres receive the impulses from a natural source, in the other case from a wholly unnatural source and in a manner different from any occurrence in the course of their normal functioning. Conceivably, the individual impulses arising in the neurones from reflex excitation are qualitatively different from those artificially induced and rise to their full intensity more gradually. Such a view is quite at variance with the conception of the nerve impulse which has developed from the researches of Lucas, Hill and Adrian.[37] Their results make the propagated disturbance in nerve appear to be a sort of explosive event whose character is always the same by whatever agent evoked, so long as the condition of the nerve remains unchanged. It further appears, so far as individual fibres are concerned, to obey the "all-or-none" law; that is, its magnitude is always the same in normal fibres regardless of the strength of the stimulus. On the other hand, it is to be remembered that all these researches on the properties of the nerve impulse have been carried out with artificial stimuli, and it is still conceivable that the reflex centre can induce in nerve fibres a different kind of activity which we have found no means of duplicating.

(2) The more gradual rise of the electrical disturbance in the case of the reflex may be quite as easily explained in a way which

[37] Lucas: Journal of Physiology, vol. 39, 1909, p. 331; ibid., vol. 40, 1910, p. 225; ibid., vol. 41, 1910, p. 368; ibid., vol. 43, 1911, p. 46; Proc. Roy. Soc., vol. 85B, 1912, p. 495. Hill: Journal of Physiology, vol. 40, 1910, p. 190. Adrian: Journal of Physiology, vol. 45, 1912, p. 389; ibid., vol. 47, 1914, p. 460; ibid., vol. 48, 1914, p. 453.

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harmonizes perfectly with the view that the impulse is essentially the same however evoked. It has already been suggested that we have no grounds for the conclusion that in the flexion reflex the impulses in the many neurones making up the motor nerve are discharged "in a volley" rather than in "platoon fire," to use Brucke's phrase.[38] They might conceivably start down the nerve trunk simultaneously in all the fibres. Yet it is quite as likely, if not even probable, that the reflex times in the hundreds of separate arcs will not be exactly the same, and that the arrival of the various outgoing impulses at a given point in the nerve will be spread out over a considerable period of time. Just such a scattering in time would perfectly explain the more gradual development of the observed electrical disturbance at the point where it is recorded. It would also contribute another factor to account for the greatly reduced intensity of disturbance as compared with the direct response; for if at any given instant only a small percentage of all the fibres taking part in the reflex are at the height of their activity, at no time will there be so great a disturbance as if all were active at once. This consideration taken in connection with those already mentioned, namely, the fact that nearly half the fibres involved in the direct response are afferent and the fact that by no means all of the motor fibres are called into action by stimulation of a single afferent nerve, may well account for the smallness of the action current obtainable from the maximal reflex.

D. The diphasic response

It was hoped that by recording the reflex response with diphasic leads (i.e., with both electrodes on the uninjured nerve trunk) some light might be thrown on the question just raised, namely, whether the reflex discharge is a "volley" of impulses which individually are different in kind from those induced by direct stimulation, having a more gradual onset, or whether it is a "platoon fire" or over-lapping series of impulses individually identical with those evoked by direct stimulation. To this end

[38] Brucke: Sitzungsberichte der Weiner Akad., 1877. See Buytendyk: Loc. cit.

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a few experiments were performed with the following change of method from that described above. A pair of shielded platinum electrodes in a glass tube on the Sherrington pattern was fastened at the entrance to the moist receiving chamber so that they made contact with the peroneal nerve close to the hip and about 3 cm. central to the proximal leading-off electrode. The nerve was carefully dissected out with all possible avoidance of trauma and laid uninjured across the boot electrodes in the chamber to render the records diphasic instead of monophasic. The secondary coil of the inductorium was wired to a switch whereby it could be connected either with the usual stimulating electrodes on the afferent nerve or with the additional pair on the motor nerve. In this way reflex and direct responses could be compared in alternation, and it could be determined whether the nerve was giving true diphasic responses under direct stimulation, an important control for the experiment at hand. After recording a series of direct and reflex responses in alternation the nerve was crushed between the leads to render the responses monophasic, and a second similar series of responses was recorded. If the nerve was long enough to permit, it was then drawn along over the electrodes till the crushed point was far enough beyond the distal lead to render the responses again diphasic. A second diphasic series was then recorded and, after crushing again, a second monophasic series.

A complicating feature of this procedure is the fact that in applying a stimulus to the motor nerve impulses are sent in two directions and a flexion reflex ensues in response to those which travel centrally in the afferent fibres of the nerve. This is revealed in a secondary action current in the record following that evoked by direct stimulation of the nerve. To show what part this reflex response played in deforming the record of the initial action current we have concluded each of these experiments by severing the peroneal nerve at the hip and then recording the response to direct stimulation uncomplicated by any reflex. Usually it was found that with a fairly tight string the record of the direct impulse was nearly complete before the reflex disturbance appeared; at any rate, the latter appeared too late

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in the record to confuse the interpretation. Records showing the results of such a series are given in figure 8. They show the events in the following order: (A) the diphasic reflex, (B) the diphasic direct response, (C) the monophasic reflex, (D) the monophasic direct response, (E) the same as (D) after cutting the nerve at the hip to eliminate the secondary reflex effect. This latter shows plainly in both (B) and (D) following the direct response after an appropriate latency.

When the attempt was made to see what light the comparison between the diphasic and the monophasic reflex responses might throw on the question which this comparison was intended to answer, it was found that the result was indeterminate. This

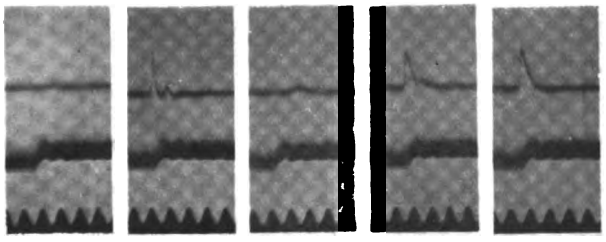


Fig. 8. Description in text. Preparation 25.
Stimulus, A, 235 Z; B, 25 Z; C, 235 Z; D, 25 Z; E, 25 Z.
String E; Tension, 1 cm. = 15×10^{-8} Amp.

was found in the following way. Hypothetical curves were plotted on coördinate paper to see what type of curve we should expect to find in each case, i.e., if we were dealing, on the one hand, with a volley of slowly developing individual impulses, or, on the other hand, with an over-lapping series of rapidly developing individual impulses. First, a single monophasic curve was drawn as a basis. This curve was not taken exactly from any known measurements, but it was given the general form known to represent roughly the rise and fall of electrical disturbance at a given point on a nerve plotted against time. For convenience the maximum was made to come 1σ after the onset, a time not far from correct. Next, by subtraction of ordinates a diphasic

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curve was made such as would result if the disturbance represented by curve 1 passed at a rate of 60 metres per second over a pair of electrodes set 3 cm. apart on the nerve, this being about the distance between the leads in most of our experiments. Next, a curve was drawn to represent, as nearly as we could estimate, the observed course of the changes in potential difference as found in the typical monophasic reflex response, the maximum being reached 3σ after the onset. Then, to show the sort of response we should expect from the diphasic lead in the case of a volley of impulses individually timed like the general response, a diphasic curve was plotted by exactly the same method of subtracting ordinates by which the direct diphasic curve was derived from curve 1, but using as a basis the reflex monophasic curve. In this it was again assumed that the impulses travelled at the rate of 60 metres per second over leads 3 cm. apart. This curve will be designated the "volley reflex diphasic curve."

Next, the effect to be expected of a "platoon fire" or overlapping series of impulses was examined. A series of six monophasic curves, each similar to curve 1,[39] was plotted, and each was made to begin 0.5σ after the preceding curve. The summed or resultant curve provided a fair counterpart of a series of impulses evenly distributed over a time interval of 2.5σ. The general contour of this curve was practically identical with the curve empirically drawn to represent the apparent potential changes in the monophasic reflex response, and its maximum was reached at the same time, 3σ after the onset. Finally, a curve was plotted on the same principle to show the presumable counterpart of a "platoon fire" or overlapping series of diphasic individual impulses. Here again six component curves, each beginning 0.5σ after the preceding, were combined, but this time each was a diphasic curve timed like a direct diphasic response. The resultant curve was found to be almost indentical with the "volley reflex diphasic curve." The differences between them were so slight that it would be clearly impossible

[39] The ordinates of these were reduced throughout to one-quarter of their values in curve 1.

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to infer from the diphasic reflex records whether they represented electrical changes of one type or the other. A similar series of curves in which a velocity of 30 metres per second throughout was assumed yielded essentially the same result. In short, unless we assume the rate of propagation of impulses along the nerve to be slower in the reflex response than in the direct, which is a most improbable assumption, we should expect to find no material difference in the diphasic records whether the slower development of the monophasic disturbance in the reflex resulted from slower development of the disturbance in individual impulses, or from the scattering in time of impulses which are individually as abrupt as those evoked by direct stimulation. The records of diphasic and monophasic responses from the same preparation, although they fail to settle the point at issue, are reproduced in figure 8 for whatever interest there may be in them.

Fortunately the answer to the question raised was accidently found in an experiment in which the animal investigated failed to yield typical reflex responses with the galvanometer. The excursions were abnormally slight and brief. It is probable that only a small proportion of the usual number of motor nerve fibres were involved in the response. The reflex records obtained by the diphasic method (i.e., before the nerve was crushed between the leads) showed clearly marked disphasic responses whose time relations were almost identical with those of the control records of diphasic responses from the same nerve stimulated directly. One of these diphasic reflex responses is shown compared with a control record and a calibration curve in figure 9. The monophasic responses obtained later from the same nerve were also exceptionally small and brief. In the diphasic reflex it may be seen (and it was confirmed by measurements on the film) that the times from the beginning of the first phase to the beginning and summit of the second phase are little if any longer than in the direct response. It appears from this that when few enough fibres respond to reflex stimulation the gradual onset of the electrical disturbance, usually so characteristic of the reflex, disappears. From this it may be fairly concluded that the individual impulses in the nerve fibres have the same time

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relations in reflex action as under direct stimulation; that is, the electrical disturbance rises to its maximum in each fibre just as abruptly whether the impulses arise in the centre or from direct stimulation. In short, there is no valid reason for assuming any qualitative difference between impulses arising from natural and from artificial sources.

E. Dicrotic reflexes

The monophasic records of reflex responses shown in the figures already referred to have all at least had this in common, that only

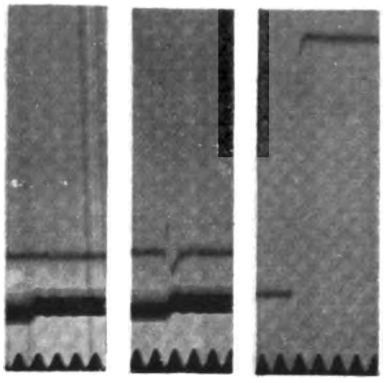


Fig. 9. Description in text. Preparation 23. String E. Stimulus, A, 164 Z; B, 41 Z; C, $\frac{50}{\text{String} + 50{,}000}$

a single excursion of the string occurred in each response. This was not always the case. In a few preparations the response with the usual monophasic leads showed two fairly distinct summits. Records from two of these preparations are shown in figure 10. Such records do not necessarily show, what might at first be inferred, that any neurones involved in the reflex respond twice. It is quite possible and perhaps more probable that this apparent doubling of the response is merely an exaggerated case of the scattering in time of the discharge in the many neurones that make up the nerve trunk. It seems quite likely that in these cases the many hundred individual reflex arcs have

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transmission times not evenly grouped about a mean, but falling distinctly in two groups. Thus, the first summit would indicate that the majority of the quickest arcs were responding, and the second summit would correspond with the reflex time of a second

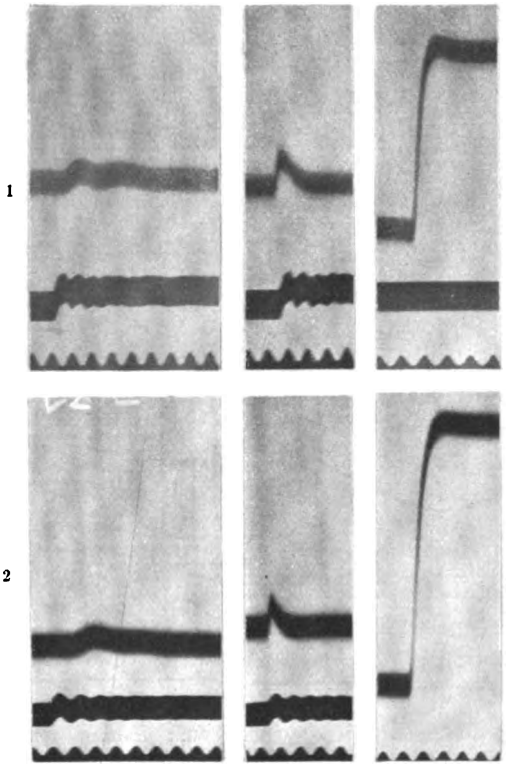


Fig. 10. String D. No. 1, Preparation 11.
Stimulus, A, 94 Z; B, 94 Z; C, $\frac{10}{\text{String} + 30{,}000}$
No. 2, Preparation 18.
Stimulus, A, 59 Z; B, 95 Z; C, $\frac{10}{\text{String} + 22{,}000}$

set of arcs. Of course the possibility that some of the neurones discharge twice is not excluded, but the other explanation seems to us the more likely.

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F. The muscular response

To compare the reflex response in the flexor muscle with that of its motor nerve the following method was used. After dissecting the peroneal nerve the tibialis anticus muscle was exposed and two sutures passed through its substance; one close to the entrance of the nerve, the other near the tendinous end. Two bits of twine soaked in Ringer solution were tied round the porous portions of the boot electrodes and their ends tied firmly in contact with the muscle by means of the sutures. These were arranged to localize sharply the points of contact and to minimize the shifting of the contacts when the muscle contracted. It is believed, therefore, that the electrical disturbance due to such shift as may have occurred is insignificant. When a series of responses from the contracting muscle under reflex stimulation had been recorded, the nerve was crushed and cut as already described, and another series of responses recorded from the nerve.

In three experiments the motor nerve was cut far enough from its entrance to the muscle to permit electrodes to be applied for stimulating the muscle directly through its motor nerve. Then, while the leading-off electrodes were still in place on the muscle, records were obtained of the action current when it was stimulated in this way for comparison with its reflex response. After this was done, leading-off electrodes were applied as usual to

Fig. 11. Comparison of reflex responses from tibialis anticus muscle (A) and peroneal nerve (B). String D used in all.

No. 1. Preparation 12.

Stimulus, A, 26.5 Z; B, 144 Z; C, $\frac{20}{\text{String} + 34{,}000}$

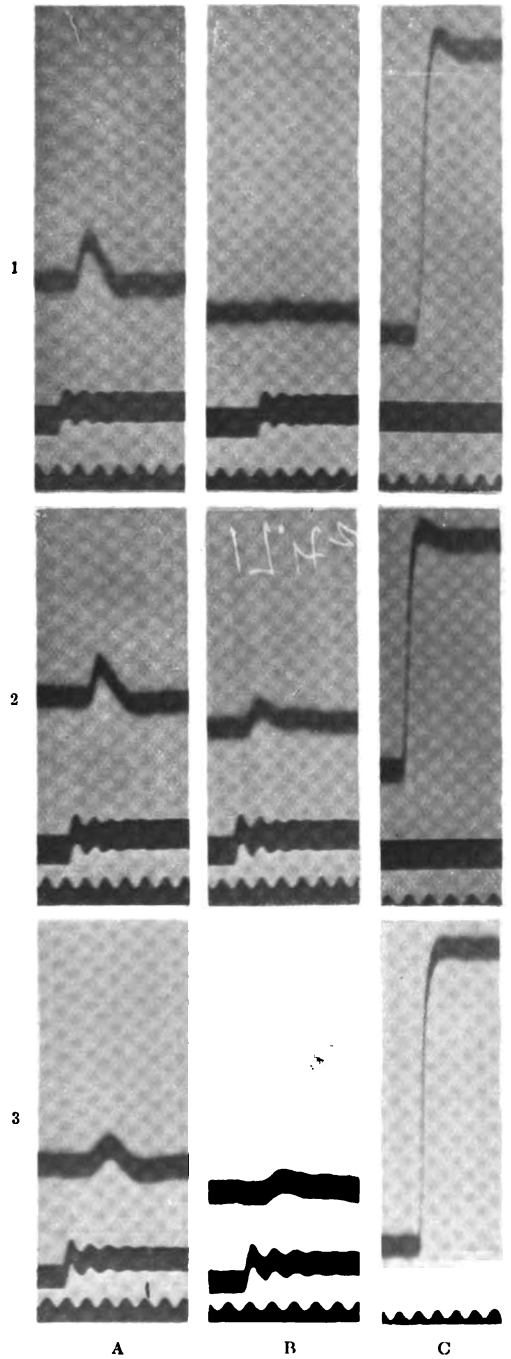
No. 2. Preparation 13.

Stimulus, A, 19.6 Z; B, 17.4 Z; C, $\frac{20}{\text{String} + 20{,}000}$ with about half the magnetic field used in the experiments. The curve is the same as Fig. 6, No. 6C.

No. 3. Preparation 18.

Stimulus, A, $\frac{272}{K}$ Z; B, $\frac{272}{K}$ Z. C, $\frac{10}{\text{String} + 19{,}000}$

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the nerve and its reflex response recorded for comparison with that of the muscle.

The method used for leading off from the muscle, i.e., exposing it in order to bring the electrodes directly in contact with the muscle substance, is not wholly free from objections. It undoubtedly has the advantage of localizing the points where activity is studied, and of leading more of the action current through the galvanometer than is possible if electrodes are applied to the intact skin over the muscle. On the other hand, exposure to the air certainly causes rapid impairment of the physiological state of the muscle. This impairment seemed to be more marked in the late autumn when the air in the laboratory was dried with steam heat than in the late spring when the air was comparatively moist and warm. In one experiment (No. 26) a combination of the method described above and that of Buytendyk was employed. Instead of exposing the whole muscle two small openings were made in the skin over the desired points and at these points strips of cloth, tied around the porous parts of the boot electrodes, were also tied to the surface of the muscle by sutures passed through its substance. Especial care was taken to avoid exposure of the motor nerve at its point of entrance to the muscle. This procedure was clearly justified by the results for the responses to both reflex and direct stimulation were notably larger than those obtained in previous experiments under otherwise identical conditions (cf. figs. 12 and 13).

The electrical responses of the muscle under reflex stimulation were found to vary far more than those of the nerve. Figure 11 shows reflex muscle responses (A) from three preparations compared with reflex monophasic responses from the motor nerves (B) recorded shortly afterwards. Figure 12 shows, in addition, responses from both muscle and nerve under direct stimulation (i.e., stimulation through the motor nerve in the case of muscle). The calibration curves in both figures refer to the response of the muscle and not of the nerve, i.e., the substituted resistance was that of the circuit including the muscle. The difference is not of great moment, for though the muscle always had less resistance than the nerve, the difference was not enough

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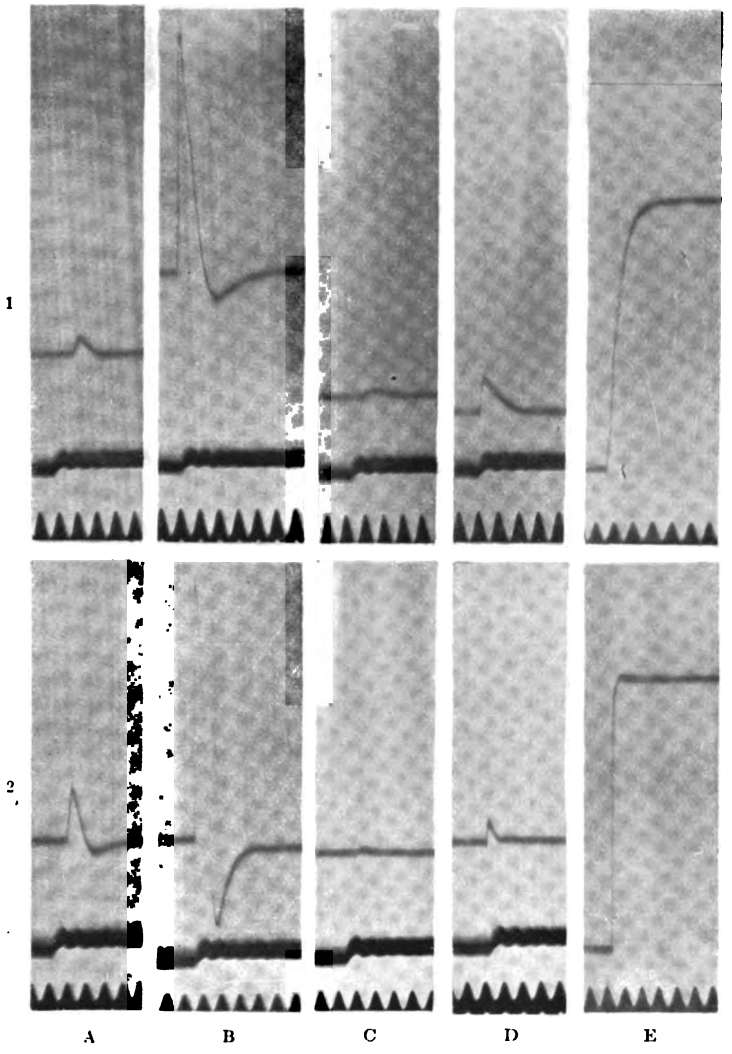


Fig. 12. *A*, reflex muscle response; *B*, response of muscle to stimulus applied to motor nerve; *C*, reflex nerve response; *D*, response of nerve to direct stimulation; *E*, calibration curve for muscle responses. All stimuli maximal. String *E* in both series. No. 1 Preparation 22. Whole muscle exposed.

Stimulus, A, 158 Z; B, 18 Z; C, 93 Z; D, 79 Z; C, $\frac{10}{\text{String} + 25{,}000}$

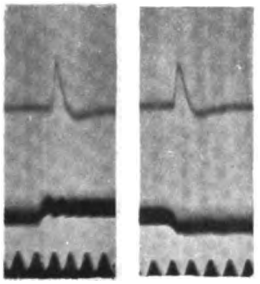
No. 2. Preparation 26. Muscle exposed only under electrodes.

Stimulus, A, 93 Z; B, 41 Z; C, 334 Z; D, 25 Z. C, $\frac{25}{\text{String} + 15{,}000}$

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to alter the shape of the calibration curve to any great extent. The three muscular responses shown in figure 11 appears to indicate simple twitches. In this they resemble the records shown by Jolly in figure 5 of his paper.[40] His records were obtained by essentially the same method, except that he stimulated by sudden pricking of the skin on the foot, and led off through the intact skin over the muscle.[41]

Although the majority of the reflex responses we haverecorded from muscle seem to have the character of a simple twitch, and in particular figures 12 and 13 show a reflex response whose time relations (excepting the latency) are nearly the same as the direct response, still this simple character is by no means universal.



A B

Fig. 13. Preparation 26. Response of muscle to maximal reflex stimulus (*A*) compared with response of same muscle to submaximal stimulus applied to motor nerve (*B*). Stimulus; *A*, 93 *Z*; *B*, make shock at same coil distance as in fig. 12, 2B, where break shock = 41 *Z*. For calibration curve see fig. 12, 2E. String E.

Considerable variety in the shapes of the curves are often found. In this respect the muscular responses differ strikingly from those of the nerve, for although considerable differences may be found in the records obtained from the nerves of different preparations, yet in a single preparation the responses almost invariably present the same character throughout the entire course of the experiment, often over an hour; what changes develop are almost always confined to magnitude and do not concern the time relations. That is, the responses may become larger or smaller but the curves still have the same general shape irrespective of the strength of stimulus. In the response from the

[40] Jolly: Loc. cit.

[41] In comparing the records it should be remembered that while his magnification was precisely the same as ours the speed of his plate was much greater, and his curves differ in shape from ours accordingly.

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muscle, however, marked difference may be found between two successive records taken within a few seconds of each other. Figure 14 shows two records, A and B, taken within half a minute of each other from a single preparation under identical conditions and with the same strength of stimulus; the difference between them is fairly marked. Figure 15 shows a series of five muscular responses from a single preparation (the same as that which furnished fig. 12, row 2) all taken within eleven minutes and without change of experimental conditions other than in the strength of stimulus. Within five minutes of the last of these the record

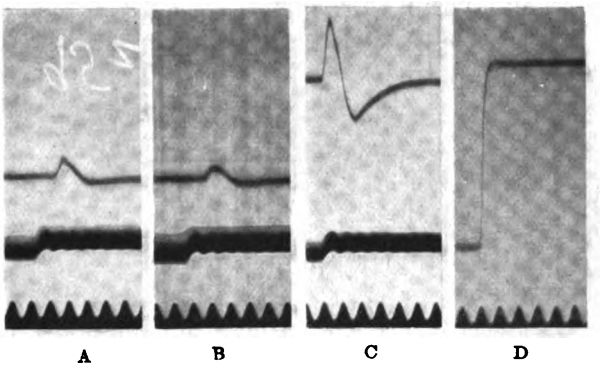


Fig. 14. Responses of muscle, Preparation 25. *A* and *B*, stimulus, 95 *Z*, reflex. *C*, stimulus, 25 *Z*, applied to motor nerve.

D, $\frac{25}{\text{String} + 30{,}000}$. String *E*.

shown in figure 12, 2A, was obtained still under the same conditions. Here, then, is a series of six records of muscular response in the flexion reflex under identical conditions and with stimuli all presumably maximal or nearly so, yet no two of them show the same shape of curve.

These observations have a bearing on the question which has been discussed by Buchanan, Piper, Garten and Dittler, whether a muscle records faithfully the impulses it receives from its motor nerve as discharged from the centre, or whether it may, under certain conditions, respond with a rhythm of its own. Of course it is not safe to base final conclusions as to what happens in pro-

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longed tetanus from observations on so brief an event as the flexion reflex, and yet these observations may be suggestive in this connection.

The interpretation of dicrotic reflex responses from the muscle presents the same problem as the dicrotic response occasionally occurring in nerve. It may be that a second propagated disturbance passes over some of the fibres after they have responded once. Or it may be that the latencies of a considerable proportion of the fibres are so much longer than that of the majority that their response is marked by a distinct notch in the curve. This latter view is difficult to reconcile with the fact that of two successive reflex muscular responses to identical stimuli one may

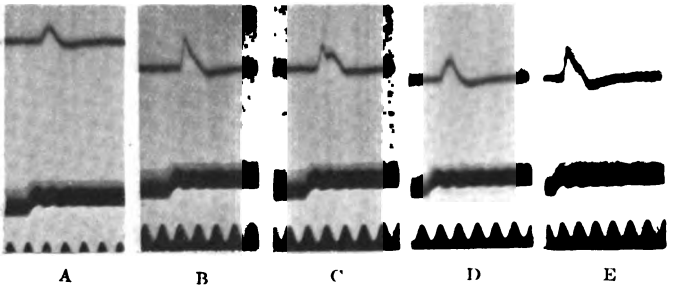


Fig. 15. Reflex responses of muscle. Preparation 26. Stimulus: *A*, 41 *Z*; *B*, 93 *Z*; *C*, 120 *Z*; *D*, 59 *Z*; *E*, 164 *Z*. For calibration curve see fig. 12, 2E.

be single and the other double. It is hard to see how the time required to evoke a reflex response in a certain group of muscle fibres could change appreciably in a few seconds without change of experimental conditions. Unless such a change of latency occurs, the only apparent explanation of the observation is that when the curve is dicrotic some of the fibres have responded twice. If this is the case our evidence supports the view that contracting muscle fibres may develop an intrinsic rhythm of response independent of the rhythm of the nerve impulses by which they are excited. It should be noted that we have not excluded with absolute certainty the possibility that the break in the primary current was not perfectly clean, and, consequently, that there may have been three shocks instead of one. That this could

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be the case without deformation of the signal magnet curve was clearly shown when the defect already alluded to in the knife-blade key gave rise to double impulses in a nerve under direct stimulation. On the other hand, all the records shown in figures 14 and 15, and several others showing dicrotic responses, were obtained with the other key (in which a sharp amalgamated copper point was withdrawn from mercury), and this key never evoked anything but single impulses from nerves directly stimulated, although several hundred such records were made with it.[42] It should also be noted that the reflex response subsequently recorded in the nerve, shown in figure 12, 2C, is apparently single, although its smallness suggests that part of the nerve may have become irresponsive. In view of these facts, it seems to us fair to assume that the break shocks were single and to conclude that either some individual reflex arcs, including the muscle fibres have latencies which vary strikingly from minute to minute, or that some if not all of the fibres in the muscle under observation may respond twice in the flexion reflex as evoked by a single shock applied to the motor nerve, although apparently only a single impulse has travelled down each motor nerve fibre. In either case, the fact that a dicrotic record is obtained from the muscle when its motor nerve is yielding single excursions of the string shows that the muscle response may be deceptive as regards rhythm of innervation.

G. Summation in the muscular response

One peculiarity of the reflex muscular response, which was not apparent in that of the motor nerve, was its great intensification under the effect of rapid summation. This was brought out by the defective breaking of the primary current already mentioned. Figure 16 shows six reflex muscular responses from two preparations, all but No. 2A obtained with the knife-blade key at a time when its operation was found to be defective in a majority of

[42] Exceptions to this statement in the case of extremely powerful shocks will be discussed in a second paper. They have no bearing on the present argument.

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makes and breaks. The lower row is from the same preparation (No. 13) that furnished the records in the second row of figure 11, and the first (A) of that series is reproduced again in figure 16, 2A. The latter, being a response to stimulation with the copper point key, shows the effect of a single stimulus. The first record in the upper row of figure 16 also shows a response to a stimulus which, though produced by the knife-blade key, was probably single, judging by the size and shape of the curve. Each of the four other records in figure 16 is almost certainly the response to a rapid succession of stimuli. In the upper row the third response is shown to be so by the signal magnet, while in the second, although the signal magnet shows only a simple make in the primary circuit, the stimulus was probably not simple. This probability is based on two considerations. One is the small preliminary excursion shown by the convexity upward preceding the main excursion in this record and in several other make shock records from this preparation, but not found in those evoked by break shocks; the other is the fact reported by Erlanger and Garrey[43] that even where the make of the primary current is clean the motion of the lagging armature of a signal magnet may modify appreciably the induced current in the secondary coil. Our signal magnet, though responding with great rapidity on the break, lags considerably on the make. We have, then, definite reason to infer that two of the four records in figure 16 showing exaggerated responses are the result of compound stimulation, and it is extremely probable that the other two owe their magnitude to the same cause, shocks coming in rapid succession instead of singly.

It is well established that summation plays a notable part in reflexes in general. Sherrington[44] has emphasized this, and Adrian and Lucas[45] have discussed it in an important paper in which they point out that in reflexes we are dealing with a "summation of propagated disturbances" which must be clearly distinguished from "summation of inadequate stimuli." The

[43] Erlanger and Garrey: Loc. cit., p. 388.
[44] Sherrington: Integrative Action of the Nervous System, 1906, p. 36.
[45] Adrian and Lucas: Journal of Physiology, vol. 44, 1912, p. 68.

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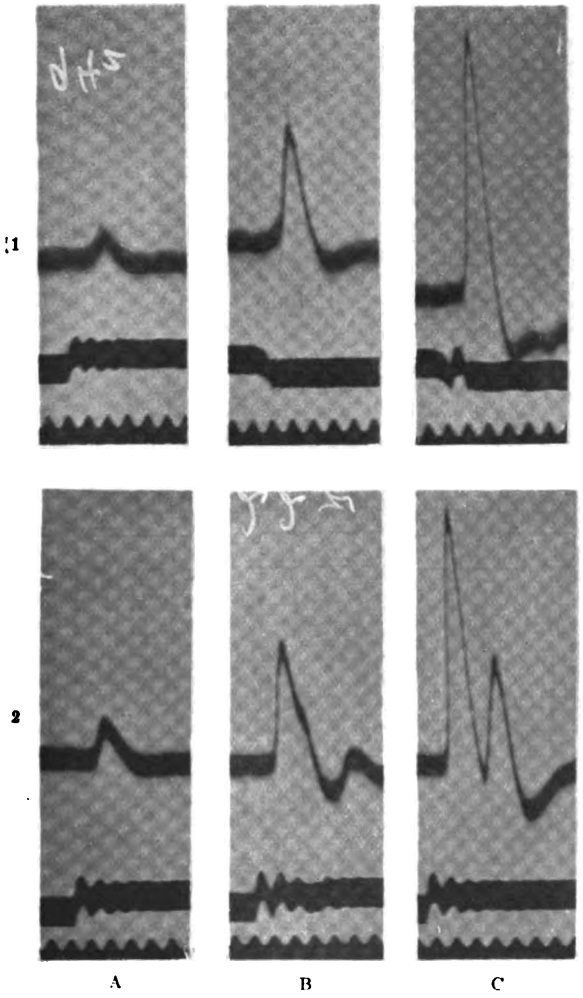


Fig. 16. Description in text. String *D*.

No. 1. Preparation 11. Stimulus; *A*, 94 *Z*. *B* and *C*, coil distances such that simple break shocks = 526 *Z* and 260 *Z*.

No. 2. Preparation 12. Stimulus; *A*, 19.6 *Z*. *B* and *C*, coil distance to give break shocks = 22 *Z*.

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magnification of the reflex response under the influence of summation is in the case of the muscle very striking. In the responses from the nerve no such pronounced intensification has been recorded. Unfortunately, we have had no way of knowing positively whether the make or break in a given record was absolutely clean or not except in those cases in which the secondary closure was long enough to reveal itself in the signal magnet record. However, a long series of reflex nerve responses was recorded from each of the two preparations furnishing the records in figure 16, and almost all of these conformed very closely to the type which in each case seemed to be characteristic of a

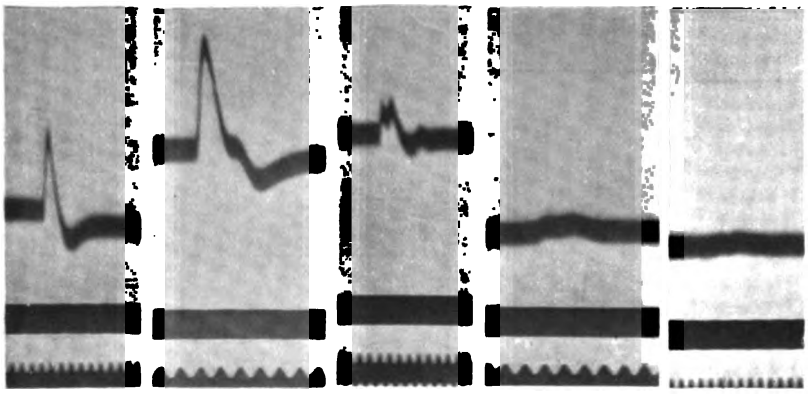


Fig. 17. Acoustic flexion reflexes recorded from muscle (*A*, *B*, *C*) and motor nerve (*D*, *E*). Preparation 12. String *D*, tension, 1 cm. 12.7×10^{-8} amp. For calibration curve see fig. 11, 2C. Stimulus, whistle; pitch higher in *A* and *B* than in *C*.

N.B. *A*, *C* and *E* were taken at half the usual speed of film.

single stimulus (see No. 6A in fig. 6 and No. 1A in fig. 10). It is eminently probable that the imperfection in the action of the key which appeared in a large proportion of makes and breaks both in the muscle series before, and in the series of direct nerve stimuli after, was also present in many cases in the long series of reflex nerve responses recorded between the two from both preparations. In one or two reflex nerve responses appreciable but slight intensification appears, but on the whole they show a

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surprising uniformity as compared with those derived from the muscle under presumably similar conditions of stimulation. This comparison, although lacking a wholly adequate control, serves to emphasize further the lack of exact correspondence already noted between the responses of the flexor muscle and of its motor nerve under reflex stimulation.

H. Acoustic flexion reflex

In a recent paper[46] acoustic reflexes in the decerebrate preparation have been reported. In Experiment VIII of that series hip flexion was found to follow acoustic stimuli. This is unusual, the response being more commonly confined to the muscles of the pinna, neck and tail. In preparation No. 13 of our series (not reported in the paper on acoustic reflexes) the same response occurred to a more marked degree. A shrill whistle evoked a sharp twitch in the trunk and limb muscles in which limb flexion appeared dominant. As this preparation was being used for a comparative study of nerve and muscle responses in the flexion reflex, these responses to acoustic stimuli were recorded electrically from both nerve and muscle in addition to those obtained by electrical stimulation. The latter have been shown in figures 6, 11 and 16. Figure 17 shows three responses to acoustic stimuli from the muscle and two by the usual monophasic leads from the motor nerve. In every case the stimulus consisted in a short, sharp whistle made by one of us near the animal, care being taken not to blow on the animal to avoid the confusion of a mechanical stimulus. It is interesting to note that while one response from muscle appears to indicate a simple twitch, all the others are dicrotic.

I. Reflex fatigue

The question of fatigue in the reflex arc has been discussed by Sherrington,[47] Lee and Everingham,[48] and others. One of us

[46] Forbes and Sherrington: Loc. cit.
[47] Sherrington: Op. cit., p. 218.
[48] Lee and Everingham: This journal, vol. 24, 1909, p. 384.

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has shown[49] that in the flexion reflex some part of the reflex mechanism is readily fatigued, but that such fatigue does not involve the motor centre as a whole. The fatigue seems confined to the particular path of approach, presumably, according to Sherrington's view, a set of synapses.

In our experiments fatigue has been found to be strikingly manifest and rapid in its development. Records illustrating this have been secured from the majority of our preparations by operating the make and break key rapidly several times by hand while the film was running continuously at second speed (13–14 cm. per second). A record of this procedure is shown in

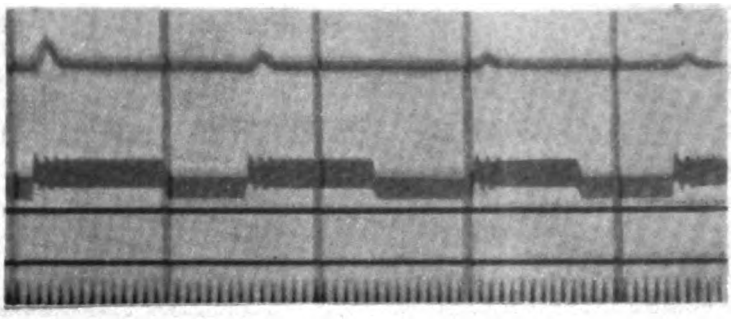


Fig. 18. Preparation 2. String *C*. Same tension as in fig. 5, No. 1. Stimulation from uncalibrated coil, apparently maximal. Responses from motor nerve showing reflex fatigue, see text.

figure 18. Here the coil distance was such that only break shocks were effective. In this instance it may be seen that the second response gave about half as large an excursion as the first, and the third excursion is only slightly smaller than the second; the fourth shows no further decrement. In one or two other cases slight decrement occurred between the second and third responses, but in the majority the result was such as is shown in figure 19. Here it will be seen that as in figure 18 there is a marked decrement between the first and second responses after which no further change occurs; a lower level is reached after the first

[49] Forbes: This journal, vol. 31, 1912, p. 102.

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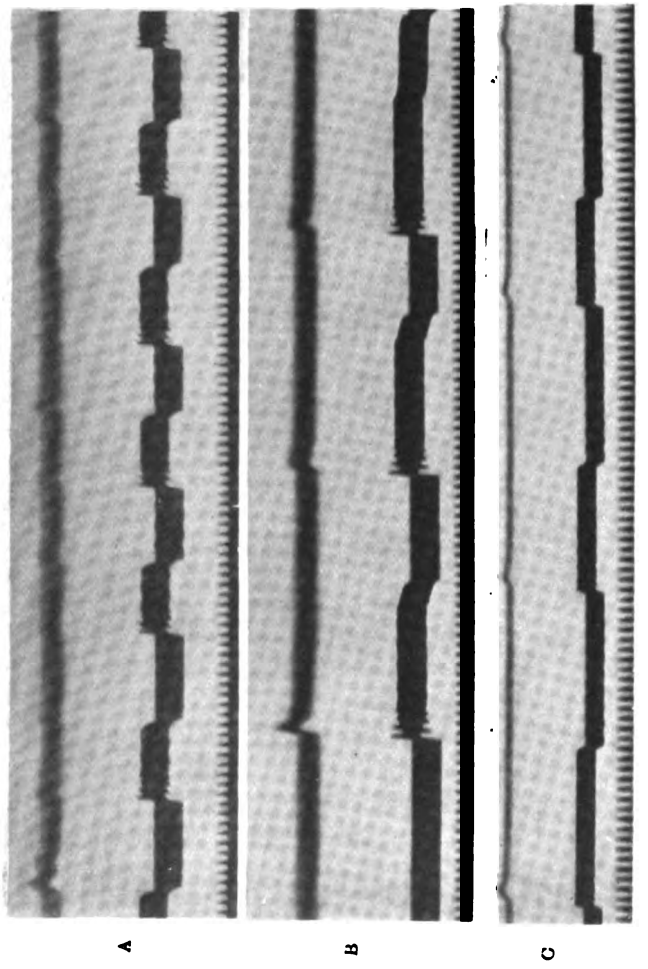


Fig. 19. Procedure as in fig. 18. *A*, Preparation 11. String *D*. Stimulation with platinum contact spring key, breaks shocks = 1560 *Z*. *B*, Preparation 13. String *D*. Stimulation with amalgamated copper and mercury key, break shocks = 22 *Z*. *C*, Preparation 20. String *E*. Stimulation with same key as in *B*, break shocks = 93 *Z*.

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response and this remains practically constant. Figure 19, A, obtained with a simple spring platinum contact key, which could be worked very rapidly by hand, shows this approximate constancy of the fatigued response over a fairly long series. In two preparations only the first stimulus produced any clearly visible response. This condition is illustrated in figure 20.

In connection with these observations it should be noted that even when the preparation is rested the magnitude of the excursions are not always constant. Occasionally a response will be below par after the usual rest and with no apparent cause. One record obtained from the same preparation that furnished figure 19, C, shows a second response greater than the first. Here the first response was sub-normal, while the second was almost as large as one obtained just before from the preparation when

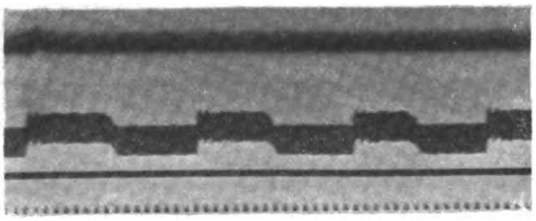


Fig. 20. Preparation 9. Apparatus and procedure as in fig. 19, A. Break shocks = 17.6 *Z*.

rested. The reduced size of the first response may have been caused by an accidental stimulus which may have occurred just before the observation was begun. The increased size of the second is puzzling as it stands practically alone among a large number of observations. The time interval was, however, longer than was usual between successive stimuli in these fatigue experiments and appears in this case to have sufficed for partial recovery.

In spite of this anomalous observation the uniformity of the responses following a rest of ten seconds or more was so nearly perfect and the regularity with which the results of the fatigue experiment conformed to the type shown in figure 19 was so general that we can safely accept the usual findings as valid.

The fact that the reflex arc fatigues so rapidly to a certain point and then fatigues no further during the course of the pro-

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cedure employed, suggests the possibility that there are two component parts of the mechanism involved, that one part is highly susceptible to fatigue and the other highly resistant. What such component parts of the reflex mechanism might be is a matter of guess-work. If we accept the "all-or-none" view of the nerve impulse held by Adrian,[50] provided each neurone responds with a single impulse in the flexion reflex, there can be no reduction in the intensity of response in the individual motor fibres through fatigue, when the interval between stimuli is as great as in our experiments. Such reduction might occur at the synapse or in the cell body, but if a propagated disturbance passed from the latter into the axon it would, according to Adrian's view, at once attain its full intensity. This view holds that the only condition under which the impulse could continue

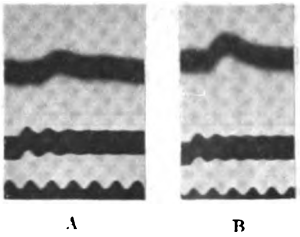


Fig. 21. Preparation 18. String *D*. Reflex responses of motor nerve. *A*, Right leg, 6 hours after decerebration, 118 *Z*. *B*, Left leg, about 24 hours after decerebration, 61 *Z*.

subnormal would be its initiation during the relative refractory period, and this in the case of the amphibian nerve at 15°C. is found by Adrian and Lucas[51] to last 0.01 to 0.02 second after the stimulus. The interval we are dealing with is often 0.25 second or more. If, then, the typical flexion reflex involves but a single impulse in each motor neurone, our observations on fatigue, to accord with the "all-or-none" view of the nerve impulse, must mean that some neurones cease to respond while others continue. If we take this view, it would further appear from our experiments that the motor neurones taking part in the flexion reflex are divided sharply in two groups, one group succumbing rapidly to fatigue, the other group continuing to respond through a long and rapid series of stimuli.

[50] Adrian: Journal of Physiology, vol. 47, 1914, p. 460.
[51] Adrian and Lucas: Loc. cit.

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We do not feel that this inference can be taken as established, for the assumptions on which it rests may be open to question. We are not certain that in the normal flexion reflex each neurone responds with a single impulse, and if the response is more than that the problem is far more complicated. Furthermore, we do not feel sure that the "all-or-none" view of the nerve impulse is established beyond question, although Adrian's evidence is hard to explain on any other basis. Considering the various elements of uncertainty we cannot commit ourselves to any interpretation of the peculiar conduct of the reflex centre when subjected to fatigue.

A few records have been made of the responses of the muscle to rapidly repeated reflex stimuli. These show substantially the same features as those recorded from the nerve and possess none which make it worth while to reproduce them.

An important difference between the flexion reflex and certain other reflexes has been brought out by the experiments just described on reflex fatigue. If one watches the animal during a series of induction shocks repeated about as rapidly as was done in these experiments, the crossed extension reflex and others still more remote, such as motions of the forelimbs and trunk muscles which are usually not elicited by a single shock, exhibit a striking degree of development under summation. Whereas, on the second or third shock of such a series, the flexion reflex has fallen to a minimum, the crossed extension and other remote reflexes show at the same time a rapid increase in intensity reaching a maximum at about the fourth or fifth shock. That summation can occur in the flexion reflex has been shown in the records reproduced in figure 16. The summation time here, however, is far more brief than that which seems effective in the crossed extension reflex. Stimuli occurring within less than one-hundredth of a second of each other appear to cause summation in the flexion reflex, while stimuli a tenth of a second apart fail to do so, the second response showing a decrement in consequence of the first. Stimuli applied at the latter interval, however, produce effective summation in the case of the crossed extension reflex and others more remote. This fact serves to

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emphasize still further the difference in the reflex behavior of the flexor and extensor groups of muscles already pointed out by Sherrington.[52]

J. The progressive increase in reflex activity after decerebration

One more fact in regard to the reflex behavior of these preparations remains to be noted. It is frequently found that when a series of reflex responses was recorded from the nerve over a period of an hour or more, the nerve being left intact in the moist chamber, a gradual increase in the magnitude of the excursions occurred throughout the series. This may have been due to the gradual evaporation of such excess of moisture as there may have been on the surface of the nerve, such moisture providing a path of short circuit for the action currents. That this increase may have been due also in part to a true increase in physiological activity is suggested by the following considerations.

It has already been noted that the flexion reflex was usually not obtainable for more than an hour after decerebration, and was seldom vigorous until even longer than that. It may be that this increased vigor in the flexion reflex signified more than the elimination of ether from the system. Certainly such reflexes as the postural tonus of decerebrate rigidity and other reflexes of apparently proprioceptive origin appeared after the discontinuance of anaesthesia very much more rapidly than the flexion reflex.

The view that the reflexes, and especially the flexion reflex, gain steadily in vigor for many hours after decerebration is supported by the following additional facts. In the case of several of our preparations, the animal, after being decerebrated in the morning and experimented on during the afternoon, was left over night and again experimented on the following morning. In these cases we generally found that a marked increase in the reflex responsiveness to handling and to operative procedure

[52] Sherrington: Integrative Action of the Nervous System, p. 301; Journal of Physiology, vol. 40, 1910, p. 105.

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had developed during the night. In one such case the usual procedure was repeated on the second day; that is, the peroneal nerve of the other leg was led into the moist chamber and placed on electrodes for monophasic responses. In this case the obvious increase in general responsiveness was marked, but the increase in magnitude of the galvanometric excursions was scarcely less marked. Two records reproduced in figure 21 will illustrate this point. Both records were produced with maximal stimulation. The first record (A) was taken in the afternoon of the first day about six hours after decerebration; the second (B) was taken the following morning in the neighborhood of twenty-four hours after decerebration. We are not prepared to state that such an increase in activity is universal, but from the few observations we have made on this point it does appear to be general.

SUMMARY

1. An optical system is described whereby the light of a Nernst lamp can be sufficiently intensified to make possible the taking of string galvanometer records with a magnification of 550 or 600 diameters and with a velocity of the photographic film amounting to 30 or 40 cm. per second, the definition sufficing for observations having a fair degree of accuracy.

2. A recording camera is described wherewith large numbers of observations can be photographically recorded in rapid succession without any requisite adjustments between. Taken in connection with the optical system, it provides an extremely convenient and elastic outfit for physiological use of the string galvanometer, involving a minimum of distraction from the purely physiological features of experimentation.

3. With the above apparatus, supplemented by illumination with an arc lamp in a few experiments when accurate time measurements were desired, the flexion reflex was examined in the decerebrate cat by stimulating an afferent nerve in the hind leg with single induction shocks, and recording the action currents in the flexor muscle (tibialis anticus) and in its motor nerve.

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4. Typical monophasic nerve action currents induced by reflex stimulation are recorded and compared with monophasic responses in the same nerve to direct stimulation.

5. A large number of such records show an observed time elapsing between stimulus and response ranging from 7.7σ to 12.8σ. From these measurements it is estimated that the "reduced reflex time" normally lies between 3 and 5σ.

6. The reflex responses in nerve to maximal stimulation are much smaller and less abrupt in their onset than the responses to direct stimulation, i.e., the electrical disturbance increases to a maximum more gradually.

7. When the nerve is giving typical reflex responses no additional information in regard to their interpretation is afforded by the diphasic method. One preparation, giving exceptionally small and short responses, furnished diphasic responses indicating that the motor impulses in the individual fibres are essentially the same, at least as regards time relations, in the case of reflex as in the case of direct stimulation. From this it is argued that the gradual onset of the typical monophasic reflex response signifies unequal latency in the individual reflex arcs, and not a qualitative difference in the individual impulses.

8. Occasionally a preparation is found in which the motor nerve with monophasic leads responds to the usual reflex stimulus with a dicrotic or double action current. This does not necessarily mean that any neurones discharge twice; it may be readily explained as a double grouping of the reflex times in the many individual arcs.

9. When the galvanometer is connected directly with the substance of the flexor muscle instead of its motor nerve, responses to reflex stimuli are obtained which differ from those obtained from the nerve in the following respects; the excursions of the string are larger, and they lack the uniformity of the nerve responses, being far more variable in shape and magnitude and often changing from single to double or double to single within a few minutes. Whether this signifies double response in some of the muscle fibres or rapid change of latency in some of the nerve-muscle units, it shows that muscular action currents

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must be used with caution as an indicator of central nervous rhythm.

10. Great augmentation of magnitude in the reflex muscular response is found under rapid summation of afferent impulses. This is not so evident in the nerve response. It is notable that the summation time is much briefer in the flexion reflex than in the crossed extension reflex.

11. In one preparation action currents were led off from both the flexor muscle and its motor nerve in response to acoustic stimuli. This reaction is very unusual.

12. The flexion reflex as recorded in the action current of the motor nerve is subject to very rapid fatigue. After the first of a series of stimuli, the response usually falls to a reduced magnitude at which it remains without further reduction. It is suggested that there are two component parts of the reflex mechanism, one part highly susceptible, the other highly resistant to fatigue. The same series of stimuli which develops flexor fatigue simultaneously produces extensor augmentation through central summation.

13. It is common to find a slow progressive increase in the vigor of the reflex responses for many hours after decerebration, and continuing until the following day.

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EXPERIMENTS ON THE ORIGIN AND CONDUCTION OF THE CARDIAC IMPULSE

V. The Relation of the Nodal Tissue to the Chronotropic Influence of the Inhibitory Cardiac Nerves

BENJ. H. SCHLOMOVITZ, J. A. E. EYSTER AND WALTER J. MEEK

From the Physiological Laboratory of the University of Wisconsin

Received for publication, February 9, 1915

INTRODUCTION

In a preceding paper of this series[1] a theory has been presented dealing with the chronotropic action of the vagus on the heart. As a result of the experimental proof of a shift of the pacemaker within the confines of the sino-auricular node, or its removal from this node to other regions of similar ("specialized") tissue, consequent upon vagus stimulation or the action of influences of a depressing character (cold, potassium chloride), it was suggested that the normal chronotropic action of the vagus is due to its power of depressing the automaticity of the several nodal tissues in a degree proportionate to the development of this function. The work to be reported in this paper was undertaken with the desire to subject this view to further experimental test. If the vagus normally acts, so far as its chronotropic influence is concerned, mainly or entirely on the

[1] Meek and Eyster: This Journal, 1914, xxxiv, 370.

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system of nodal tissues, successive removal of this tissue from the sphere of action by depressing it below the limits of activity or by excising it, should show likewise a progressive diminution of the chronotropic influence of the vagus over the heart.

HISTORICAL

Wybauw[2] in 1910, showed by the aid of the string galvanometer, that under strong vagus stimulation the upper end of the sino-auricular node ceased to manifest initial negativity when compared with the right auricle, indicating that the pacemaker had been removed from its normal position. He made no attempt to determine the new position of the seat of impulse formation, but stated that the impulse continued to arise always within the immediate neighborhood of the node. The occurrence of so-called nodal or auriculo-ventricular rhythm under vagus stimulation, a condition in which the auricles and ventricles beat simultaneously or nearly so and which recent work has demonstrated to depend upon the assumption of impulse formation by the auriculo-ventricular node, was observed as long ago as 1904 by Lohmann.[3] This shift in the seat of the pacemaker, resulting from vagus stimulation, has been noted by various workers since this time.

Flack,[4] one of the discoverers of the sino-auricular node, in 1910 made an experimental study to determine the importance of this node to the vagus mechanism. He applied certain drugs, local cold and pressure, to the sino-auricular node of the exposed mammalian heart and determined their effect on the chronotropic influence of the vagus. Application of atropin to the node abolished the influence of the right vagus on both auricles and ventricles, while in some cases the left vagus remained temporarily effective so far as the ventricles were concerned, but lost its influence on the auricles. Muscarin applied to the node slowed the heart, at first increasing, but later abol-

[2] Archiv internat. de Physiol., 1910, x, 78.
[3] Archiv f. (Anat. u.) Physiol., 1904, 431.
[4] Journ. of Physiol., 1910–1911, xli, 64.

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ishing the influence of the vagus. It was possible to show an antagonism between the action of these two substances on the nodal tissue. In two experiments on dogs, freezing the node with an ethyl-chloride spray abolished the chronotropic influence of the right vagus while the left continued to act. Clamping the node in rabbits abolished the influence of the right vagus in six or seven experiments. The effect on the influence of the left vagus was inconstant. Flack concluded that destruction of the sino-auricular node abolished the action of the vagus which had normally produced the greatest chronotropic effect and that the node represented that part of the heart upon which the inhibitory mechanism produced its main effect on cardiac rate. In a series of later experiments, Flack[5] studied the influence of curare and nicotin applied to the sino-auricular node in dogs and rabbits. Both abolished the chronotropic influence of the vagi. Application of these poisons to various parts of the auricles and ventricles outside the region of the node were uniformly negative so far as any effect on the influence of the vagi was concerned. He reaffirmed his original conclusion that the sino-auricular node was of especial importance in relation to the inhibitory cardiac mechanism. Unfortunately, Flack's records were made with too slow a speed to determine the relations of auricular and ventricular contractions, and hence to determine any possible change in the seat of impulse formation. His results, so far as they concern abolition of the chronotropic influence of the vagus due to elimination of the sino-auricular node, are opposed to those of Rothberger and Winterberg[6] and others, who have found a lessened but definite influence of the vagi in auriculo-ventricular rhythm.

Numerous writers have emphasized the difference in distribution of the cardiac fibers of the two vagus nerves. Garrey[7] has shown the homolateral distribution of the two vagus trunks in the tortoise heart, and Rothberger and Winterberg,[8] Cohn,[9]

[5] Archiv internat. de Physiol., 1911, xi, 111, 119 and 127.
[6] Archiv f. d. gesamt. Physiol., 1911, cxli, 343.
[7] This Journal, 1911, xxviii, 330.
[8] Loc. cit.
[9] Journ. Exp. Med., 1912, xv, 49.

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Robinson and Draper[10] and Ganter and Zahn[11] have emphasized the difference in distribution of the two vagi in the dog's heart, the right affecting mainly the rate of the heart, presumably as a result of its distribution mainly to the sino-auricular node. Ganter and Zahn have further shown that warming the sino-auricular node may cause a diminution or even a complete abolition of the chronotropic vagus influence. The increase of automaticity produced by the warming presumably tends to counteract the depression of automaticity produced by the action of the vagus fibers ending in the node.

METHODS

Two groups of experiments were performed, all on dogs under ether anaesthesia. In the first group, comprising five experiments on dogs, the thorax was opened under artificial respiration, the pericardium incised and the sino-auricular node exposed. The contractions of the right auricle and ventricle were recorded upon a rapidly moving Hürthle kymographion by air transmission. Shielded electrodes on each vagus were connected through a commutator to a Gaiffe induction coil. Each vagus was stimulated for a period of five seconds (as determined by a stop watch) with a strength of current sufficient to cause a moderate degree of inhibition. The degree of vagus action under normal conditions with a certain strength of stimulus was usually determined in this way several times. The upper end of the sino-auricular node was then cooled for a period of from five to twenty seconds by the application of a pencil of ice and the vagus stimulated for five seconds with the strength of current previously employed. Comparison between the normal degree of inhibition and that after cooling gave information as to the influence of the cooling on the chronotropic effect of the vagus.

Previous experiments,[12] in which the location of the pacemaker was determined by galvanometric methods, have shown

[10] Robinson and Draper: Journ. Exp. Med., 1911, xiv, 217.
[11] Archiv f. d. gesamt. Physiol., 1912, cxlv, 337, and 1913, cliv, 492.
[12] Loc. cit.

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that cooling the upper end of the sino-auricular node is frequently associated with removal of the pacemaker to the lower part of this node. This change was invariably associated with slight shortening of the As-Vs interval and a slight increase in the length of cycle. This association was so constant that we have come to regard the changes in the As-Vs interval and length of cycle as criteria of such a shift in the location of the pacemaker second only in importance to actual proof of a change in the location of initial negativity by the use of the galvanometer. We have felt justified therefore in the present experiments in accepting these criteria as proof of such a change in the seat of impulse initiation.

In some experiments cooling was carried out by means of an ethyl-chloride spray, the action of which was carefully localized by a mask. Later, in most experiments, the whole node was cooled by the application of ice or an ethyl-chloride spray. It is to be noted that cooling the upper end of the node produced always a slight slowing, while cooling the whole node produced a still greater depression of the rate. The amount of slowing as a result of the subsequent vagus stimulation is always to be compared with the rate immediately before stimulation.

Five dogs were used in the second group of experiments, in which the upper half of the sino-auricular node, the remainder of this node and the auriculo-ventricular node were successively removed while the heart was beating under artificial perfusion through the coronary arteries. The chest was opened under artificial respiration, the heart exposed and rotated slightly to expose the sino-auricular region. Ligatures were laid around the descending aorta and the left carotid, the animal bled, and the defibrinated blood mixed with Locke's solution in the perfusion apparatus of Eyster and Loevenhart.[13] Perfusion was made through the innominate artery, the escape of fluid occurring through an opening made in the inferior vena cava. The beat of the right auricle and right ventricle was recorded by air transmission. The vagi were exposed and shielded electrodes

[13] The Journ. of Pharm. and Exp. Therap., 1913, v, 57.

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applied as in the previous experiments. The normal degree of vagus inhibition produced by a certain strength of stimulus to each vagus was now compared with the degree produced after successive removal of the regions described above.

EXPERIMENTAL RESULTS

The effect of local cooling of the upper end of the sino-auricular node

This procedure was carried out in five experiments. In the the first experiment there was no effect produced, and subsequent examination of the records showed that the heart was in auriculo-ventricular or "nodal rhythm," the auricles and ventricles beating simultaneously throughout. Since the auriculo-ventricular node and not the sino-auricular node was acting as pacemaker in this experiment, it would be expected that cooling the latter would have no influence on the rate of the heart or on vagus influence. This experiment serves well, therefore, as a control for the others. It should be noted that stimulation of the vagus in this experiment produced in each trial a positive As-Vs interval, indicating probably, as will be evident later, a transitory shift in the location of the pacemaker to the sino-auricular region.

In each of the other experiments, positive results were obtained. In the four experiments, fifteen series were tabulated in which the influence of the right vagus with a constant strength of stimulation was compared before and after cooling the upper end of the node. In twelve of these the cooling was produced by means of a pencil of ice applied to the upper end of the node for a period of from five to fifteen seconds, and in one instance thirty seconds. In three, the cooling was by means of an ethyl-chloride spray, carefully localized by means of a mask. The influence of similar cooling on the degree of left vagus influence was likewise determined in ten trials, seven in which the cooling was with a pencil of ice, three in which ethyl-chloride was used. In every case but one of both of these series, the effect of cooling was to reduce the efficiency of the vagus, as shown by the fact that the vagus, with the same strength of stimulation, exerted a lessened effect on the existing rate after the cooling than it did

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before. The effect of cooling alone in every case was a slight or moderate slowing of the heart. In most cases associated with this lengthening of the cycle, there was a shortening of the As-Vs interval. The decrease in As-Vs interval amounted usually to 0.01 to 0.04 seconds. In a few cases no appreciable change in As-Vs interval occurred. Due to the fact that the cooling alone produced a slowing of the rate, the combined chronotropic effect of cooling plus vagus stimulation was usually greater than either alone, notwithstanding the fact that the efficiency of the vagus was decreased. This occurred in seventeen of the total of twenty-five trials with right and left vagus stimulation noted above. In the eight remaining series, the efficiency of the vagus was so far reduced by the cooling that the chronotropic influence of vagus stimulation plus the cooling was actually less than vagus stimulation with the same strength of stimulus before the cooling. Examples may serve to make this difference clear.

In the first of the series of Experiment 2, right vagus stimulation, for a period of five seconds, with the coil at 6, caused a lengthening of the auricular cycle from 0.36 to 0.57 seconds (58 per cent). Cooling the upper end of the node caused the cycle to lengthen from 0.36 to 0.44. Cooling plus stimulation of the vagus for the same period and with the same strength of current now caused the cycle to lengthen from 0.44 to 0.63 (43 per cent). There was no change in the As-Vs interval as a result of the cooling. In this instance cooling plus vagus stimulation increased the cycle length from 0.36 to 0.63, while vagus stimulation alone increased it from 0.36 to 0.57. The cooling plus the vagus influence thus exerted a greater effect on the rate than either alone, but not equal to the sum of the two. The efficiency of the vagus, as measured by its effect on the existing rate was reduced by the cooling. Before cooling, it produced a reduction (an increase in the length of the cycle) equal to 58 per cent of the existing rate. After cooling, this effect was reduced to 43 per cent. In a subsequent series in the same experiment, much stronger cooling of the upper end of the node by means of the ethyl-chloride spray so far reduced the efficiency of the vagus that the effect of cooling plus stimulation of the

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vagus was less than vagus stimulation alone. The strength of the vagus stimulation was also stronger (coil at 9). Right vagus stimulation alone increased the auricular cycle from 0.48 to 1.27 (164 per cent). The As-Vs interval before the stimulation was 0.10 seconds. Cooling the upper end of the node now increased the length of the cycle from 0.48 to 0.56, and the As-Vs interval was reduced to 0.06. Stimulation of the vagus with the same strength of stimulus and for the same length of time now lengthened the cycle from 0.56 to 0.93, or only 66 per cent. The efficiency of the vagus was thus reduced about two and one-half times. The total effect of cooling plus stimulation was less than the stimulation before the cooling. The former lengthened the cycle from 0.48 to 0.93, the latter from 0.48 to 1.27. Almost complete recovery was evident within nine minutes after the period of cooling. The As-Vs interval had returned to 0.10 seconds, the cycle length to 0.41 seconds. Stimulation now lengthened the cycle from 0.41 to 1.02 seconds (149 per cent.)

Milder cooling with lower strength of vagus stimulation may cause similar changes with more rapid recovery. Thus in the first series of Experiment 3, right vagus stimulation alone, with coil at 7, lengthened the auricular cycle from 0.28 to 0.48 seconds, or 72 per cent. Cooling the upper end of the node for five seconds lengthened the cycle from 0.28 to 0.33, and this increased to 0.36 (9 per cent) on subsequent vagus stimulation. Within one minute partial recovery had occurred. The cycle shortened to 0.30, and then increased to 0.42 seconds, or 40 per cent on vagus stimulation. Cooling produced a slight shortening of the As-Vs interval, returning gradually to normal after the period of cooling. Examples intermediate between these occurred in which the reduction of vagus efficiency was such that cooling plus vagus stimulation produced approximately the same result as vagus stimulation before the cooling. Thus in the second series of Experiment 5, vagus stimulation alone, with the coil at 9.5, lengthened the auricular cycle from 0.35 to 0.56 (60 per cent). Cooling the upper end of the node with ice for fifteen seconds lengthened the cycle from 0.35 to 0.48 seconds and

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reduced the As-Vs interval from 0.10 to 0.075 seconds. Vagus stimulation as above now lengthened the cycle from 0.48 to 0.59 (23 per cent). One and a half minutes later similar stimulation lengthened the cycle from 0.36 to 0.52 seconds (44 per cent). The As-Vs interval before stimulation was 0.085 second. Three and a half minutes later the As-Vs interval had returned to 0.10 second. Vagus stimulation as above lengthened the cycle from 0.36 to 0.54 second (50 per cent).

The degree of vagus depression in any given case clearly seems to depend on the intensity and duration of the cooling of the upper end of the node. Short periods of cooling with ice tend to cause a moderate lengthening of the cardiac cycle, with no change in the As-Vs interval. Subsequent vagus stimulation shows a diminished chronotropic influence, but usually not sufficient to prevent the occurrence of a greater influence of the combined cooling and vagus stimulation than with the latter alone. Longer periods of cooling with ice, or more intense cooling with an ethyl-chloride spray, on the other hand, almost inevitably led to such a strong depression of vagus influence that the vagus stimulation combined with cooling caused a smaller reduction of the normal rate than vagus stimulation before the cooling. This is particularly evident if the strength of vagus stimulation employed is sufficient to produce a considerable slowing before the region is cooled.

A certain difference in reaction of the two vagi toward cooling the upper end of the node is to be noted. The actual effect on the left vagus of approximately the same degree and duration of cooling was usually greater than the right. In two of the ten trials, moderate cooling of the upper end of the node (in one case for five seconds, in the other for ten seconds), completely abolished the chronotropic influence of the left vagus. There is no similar result in any of the trials with the right vagus.

Figures 1 and 2 are examples of the two groups separated above. In one the vagus efficiency is reduced but not to the degree necessary to prevent the combined effect of cooling and vagus stimulation from exerting a greater slowing than vagus

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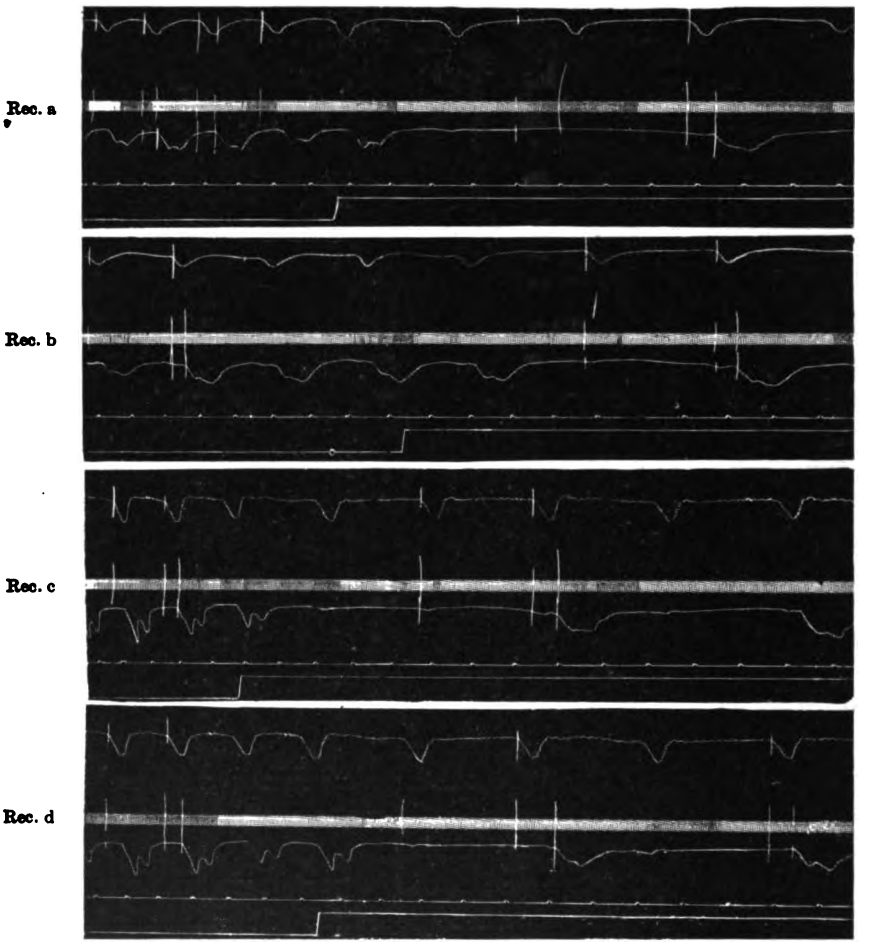


Fig. 1. *Effect of local cooling of the upper end of the sino-auricular node on the chronotropic influence of the right vagus.* The vagus influence is decreased by the cooling. The slowing produced by the combined cooling and vagus stimulation is however greater than that produced by stimulation alone.

The records are to be read from left to right. Beginning at the top of the record the first curve in each case is a record of the mechanical systole (downstroke) of the right auricle; the second curve is that made by a tuning fork vibrating 100 times a second; the third curve is the mechanical systole (downstroke) of the right ventricle; and the fourth curve is a time record with ½ second intervals. The elevation of the lowest curve records the period of stimulation (coil at 9.5 cm.).

Record a. Effect of right vagus stimulation, coil at 9.5 cm.

Record b. Same, after cooling the upper end of the sino-auricular node with a pencil of ice for 15 seconds.

Record c. Same, 1½ minutes after cessation of the period of cooling.

Record d. Same, 3½ minutes after cessation of the period of cooling.

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stimulation alone. In the other the vagus efficiency is so far reduced by the cooling that the sum of the effects produced by the two is actually less than that produced by vagus stimulation before the cooling or after the effects of the cooling had passed away.

The effect of cooling the whole of the sino-auricular node

This procedure was carried out in eleven series. In seven the influence of the right, in four that of the left vagus was studied. In all cases cooling the whole node produced auriculo-ventricular rhythm with the As-Vs interval from 0.04 second to zero. In one of the four cases of left vagus stimulation, the vagus was no longer effective after the auriculo-ventricular rhythm was established. In all the remaining series the invariable effect of vagus stimulation following the cooling was to cause a temporary return of the normal sino-auricular rhythm with the As-Vs interval equal to or even greater than the normal. The efficiency of the vagus was diminished in every case after the establishment of the auriculo-ventricular rhythm. In some instances cooling combined with vagus stimulation produced a greater effect on the normal rate than stimulation before the cooling, usually however the effect of cooling combined with stimulation produced less effect than stimulation alone. Examples of these two types may be given. In one series in Experiment 5, stimulation of the left vagus with the coil at 9.5 lengthened the auricular cycle from 0.36 to 0.62 (72 per cent). The As-Vs interval was 0.09 before and during the stimulation. Cooling the sino-auricular node with ice for fifteen seconds lengthened the cycle from 0.36 to 0.53 and the As-Vs interval became zero. Stimulation of the vagus now lengthened the cycle from 0.53 to 0.81 second (53 per cent) and the As-Vs interval lengthened to 0.07 second. A subsequent series in the same experiment will illustrate reduction in the combined effect of cooling and stimulation below that of stimulation alone. With the coil at 9.5, stimulation of the right vagus lengthened the cycle from 0.43 to 0.71 second (66 per cent). The As-Vs

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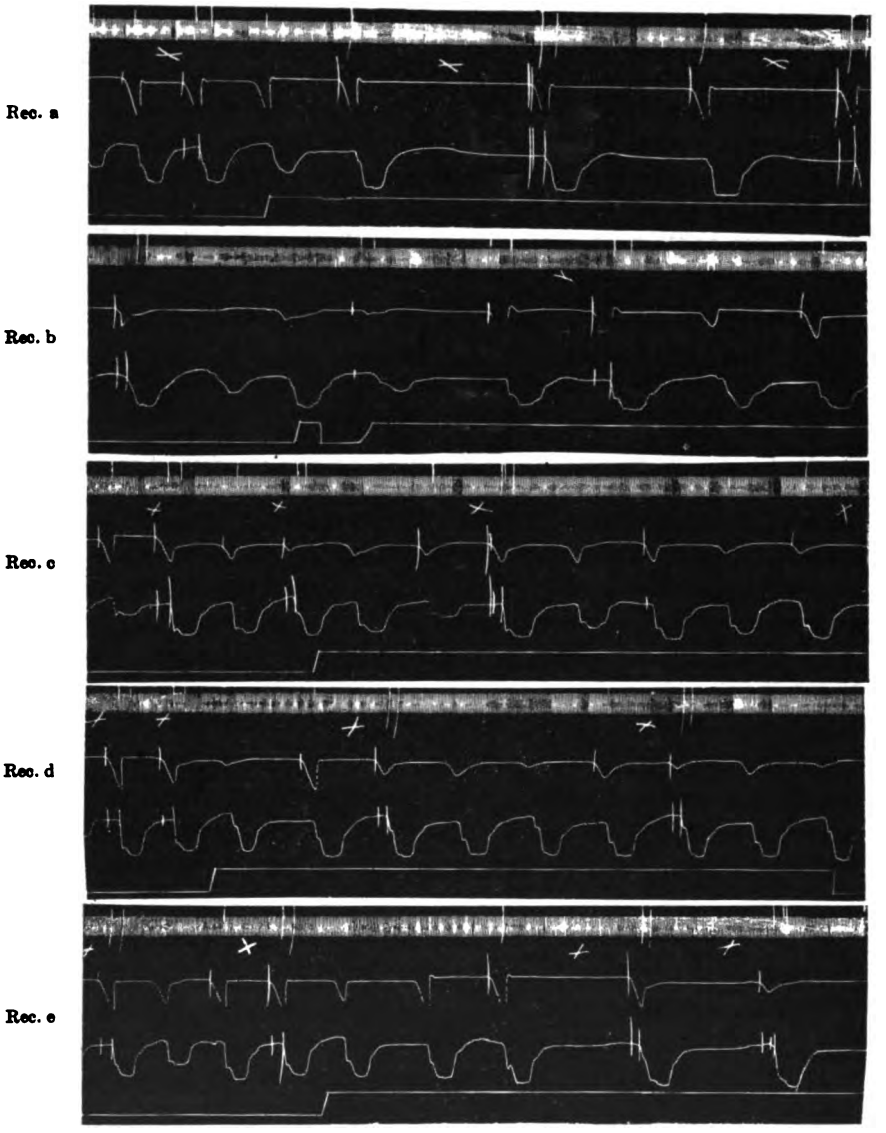


Fig. 2. *Effect of local cooling of the upper end of the sino-auricular node on the chronotropic influence of the right vagus.* The vagus influence is decreased to such a degree by the cooling that the slowing produced by vagus stimulation combined with the cooling is less than that produced by the stimulation alone.

The records are to be interpreted as in figure 1, except that there is no ½ second time record. Coil at 7 cm. throughout.

Record a. Right vagus stimulation.

Record b. Same, after cooling the upper end of the sinus node for 10 seconds with an ethyl chloride spray.

Record c. Same, 3 minutes after cessation of the period of cooling.

Record d. Same, 6 minutes after the end of the period of cooling.

Record e. Same, 9 minutes after the end of the period of cooling.

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interval was 0.09 before and during the stimulation. Cooling the sino-auricular node with an ethyl-chloride spray increased the length of the cycle from 0.43 to 0.53, and the As-Vs interval became zero. Stimulation now lengthened the cycle from 0.53 to 0.65 (26 per cent), and the As-Vs interval became equal to 0.09 second. Vagus stimulation before the cooling thus increased the length of the cycle from 0.43 to 0.71, while the combined cooling and vagus stimulation increased it from 0.43 to 0.65. One and a half minutes after the period of cooling the length of the cycle was 0.45 second and the As-Vs interval had lengthened to 0.07 second. Stimulation now lengthened the cycle from 0.45 to 0.71 (57 per cent) and increased the As-Vs intervals to 0.09 second. Three and a half minutes after the period of cooling complete recovery had occurred as was shown by the fact that vagus stimulation now increased the length of the cycle from 0.43 to 0.72 second (68 per cent) and the As-Vs intervals of 0.09 and 0.10 were present before and during the stimulation.

The effect of excision of the upper part of the sino-auricular node in the heart under artificial perfusion

Of the four experiments in which a portion, comprising approximately the upper half, of the sino-auricuar node was excised, there was definite evidence in three that this procedure diminished the chronotropic influence of the right vagus. In the fourth case this point was unfortunately not determined. A strength of stimulus was employed which gave complete inhibition both before and after removal of the upper part of the node and it was impossible to say in this case whether any reduction had occurred. The influence of the left vagus was clearly reduced in two of the four experiments, in the two other experiments this point was not satisfactorily determined. In Experiment 7, with the heart intact under artificial perfusion, the right vagus increased the length of the auricular cycle from 0.68 to 1.35 seconds (98 per cent) with the coil at 11 cm. The left vagus with the same strength of stimulus increased the cycle from 0.68 to 1.45 seconds (113 per cent). The As-Vs interval in each case

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was 0.18 second. After the excision of the upper half of the sino-auricular node, neither vagus was effective with the strength of stimulus previously employed. With a stronger stimulus (coil at 13) the right vagus increased the length of the cycle from 0.68 to 0.82 second (21 per cent); the left vagus from 0.69 to 1.83 seconds (165 per cent). The As-Vs interval was 0.16 second. With still stronger stimulation (coil at 15), the right vagus increased the length of the cycle from 0.69 to 0.92 second (33 per cent). The left vagus gave complete inhibition throughout the five second period of stimulation. In Experiment 9,

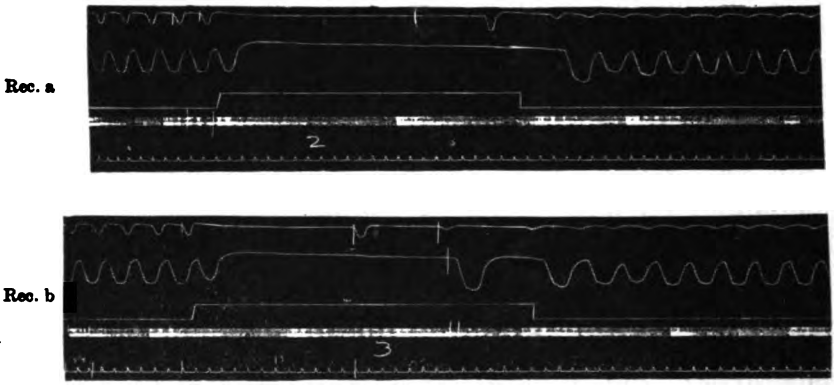


Fig. 3. Effect of excision of the upper part of the sino-auricular node in the dog's heart under artificial perfusion on the chronotropic influence of the left vagus.

The figure is to be interpreted as figure 2.

Record a. Left vagus stimulation with intact heart. Coil at 11 cm.

Record b. Left vagus stimulation immediately after excision of upper portion of sino-auricular node. Coil at 11 cm.

excision of the upper part of the node almost completely abolished the influence of the right vagus. The left produced only partial inhibition after this procedure while before it had produced complete inhibition. Excision of the upper part of the node caused in the four experiments definite shortening of the As-Vs interval, and in three also lengthening of the auricular cycle, indicating that in each case it had been serving as the seat of impulse formation. The influence of removal of the upper part of the node in Experiment 8 is shown in the records of figure 3.

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The effect of the excision of the whole of the sino-auricular node and the auriculo-ventricular node

Removal of the remainder of the sino-auricular node reduced the effect of both the vagi over that present on removal of the upper part of the node in three of four experiments. In one the influence of both vagi, while reduced by removal of the upper part of the node, suffered no further reduction on removal of

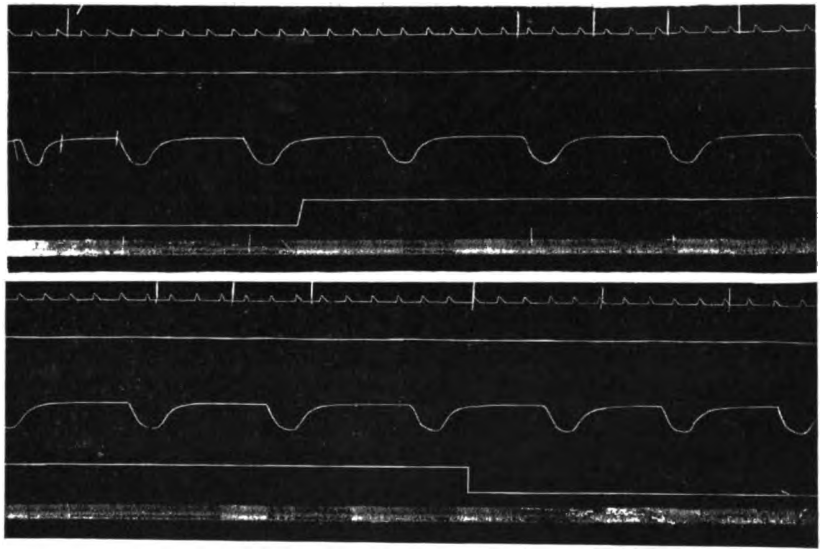


Fig. 4. One-third natural size. Shows a slight chronotropic influence of the right vagus on the ventricles in a dog's heart under artificial perfusion after removal of the sino-auricular and auriculo-ventricular nodes.

The top line of each record is the time in intervals of $\frac{1}{5}$ second; the second line is a record of the right auricle, which is quiescent following excision of the nodes; the third line shows the right ventricular beat; the fourth line the period of stimulation of the vagus; the lowest line, time in intervals of $\frac{1}{10}$ second. The lower record is a direct continuation of the upper, and shows the termination of the period of vagus stimulation begun in the former.

the remainder of the node. In this experiment subsequent removal of the region of the inter-auricular septum containing the auriculo-ventricular node abolished the influence of both vagi. In a fifth experiment the removal of the entire sino-auricular node at one time caused a reduction in the influence of both vagi.

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After removal of the sino-auricular and auriculo-ventricular nodes, the auricles stopped permanently in each of four experiments, while the ventricles in all but one continued to beat at a slower rhythm. In one of three cases in which the ventricles continued to beat it was possible to show a greatly reduced but definite chronotropic influence of both vagi (fig. 4). In the remainder it was impossible to demonstrate any chronotropic influence even with strong stimulation.

DISCUSSION

It is well known that the automaticity of the heart, measured by its rate of stimulus production, varies with the temperature. The work of McWilliams, Adam, Ganter and Zahn and others has shown that in the intact heart this influence of change of temperature is confined to that region which is at the time initiating the impulse or acting as "pacemaker" for the whole heart. Our hypothesis of the chronotropic influence of the vagus is essentially that it brings about some change in the heart which results in a depression of automaticity in the pacemaker analagous in every way to the effect of local cooling. In a previous paper of this series it has been shown that each of these influences may involve a shift in the seat of impulse formation which are similar or identical in nature. Our work has further led us to the view that within limits which approximate the normal, the seat of impulse formation is always confined to some part of the sino-auricular or auriculo-ventricular nodes. Hence the chronotropic influence either of cold or of vagus activity must be normally exerted on these regions of so-called specialized tissue. The clearest application of the experimental results reported above to a test of this general hypothesis may be perhaps given by considering in some detail each of the possible effects that these two influences, namely, cold and the vagus influence, could be supposed to produce when working singly or in combination on the rate of impulse formation of the heart. In this way it may be seen whether the experimental facts observed can be explained in terms of the hypothesis.

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We may consider two regions in the automatic tissue of the heart possessed of two different inherent powers of automaticity. These we may call A and B and consider that both lie within and represent different parts of the sino-auricular node.[14] A is by designation the region of highest automaticity. For the sake of illustration we may suppose that A has under the conditions of an experiment the power to develop rhythmic impulses at the rate of 100 per minute, B at the rate of 87 per minute. Under normal conditions, A will act as the pacemaker and will dominate B and all other regions of lower automaticity. A will continue to act as the seat of impulse formation unless some change should come about that would depress its power to discharge below that of B, or raise the power of B or some other region above it. According to the terms of our hypothesis, the chronotropic vagus influence is normally exerted in the highest degree upon the regions of higher automaticity, extending to the lower regions in a diminishing degree. A slight degree of vagus stimulation might thus depress A and B, the former more but not sufficient to cause its automaticity to fall below B. A would continue to act as the seat of impulse formation but would discharge at a slower rate. A stronger vagus stimulus might now, due to its greater influence on A than on B, actually depress the automaticity of A below B, and a shift in the seat of impulse formation result. This change we have actually observed in previously reported experiments. Our present problem is to attempt to analyze the action of an additional depressant, namely, cold, applied to A, and to ascertain how this modifies the effect of subsequent vagus stimulation. Several possibilities would seem to offer themselves, depending upon (1) the degree of reduction of automaticity produced by the cooling, (2) the strength of vagus stimulation employed. Assuming a fixed ratio between the normal discharge rate of A and B, namely,

[14] We have previously shown (loc. cit) that the lower part of the sino-auricular node may take on the function of impulse initiation at a somewhat slower rate of discharge after local depression of the upper part of the node by cold or by mild vagus stimulation.

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100 and 87, as above stated, the first possibility may be expressed as follows:

	A	B
Normal discharge	100	87
Vagus stimulation alone	90	85
Cooling of A	95	87
Cooling combined with vagus stimulation	86	85

In the above, moderate vagus stimulation may be supposed to depress the automaticity of A and B, A to a greater extent, but not sufficient to fall below B. A continues, therefore, to act as pacemaker. Local cooling of A of mild degree would likewise depress the automaticity of A, but not below B. Cooling combined with vagus stimulation would tend to produce a greater effect than either alone, but still the automaticity of A is higher than that of B and A continues as pacemaker. Thus vagus stimulation alone, cooling alone or both combined are insufficient to cause a shift in the seat of the pacemaker. The efficiency of the vagus, that is its effect on the existing rhythm, is less than before cooling, but the combined effect of vagus stimulation and cooling is greater than the former alone. There are a number of examples in our experiments which would seem to fall into the above scheme in all their details. An example is furnished by the first series of Experiment 2 quoted in the preceding section. Previous work has taught us that except for actual determination of the seat of initial negativity, the two most most important criteria of a shift in the seat of the pacemaker are (a) a lengthening of the cardiac cycle or slowed rate of impulse formation, (b) a shortening of the As–Vs interval. Mild local cooling of the upper end of the sino-auricular node may cause no change in the As-Vs interval.

A second possibility occurs associated with a somewhat greater depression of the region of greatest automaticity by more prolonged or more intense cooling. It may be represented as follows:

	A	B
Normal discharge	100	87
Vagus stimulation	90	83
Cooling of A	88	87
Cooling combined with vagus stimulation	82	⟶83

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In this case, neither cooling alone nor vagus stimulation alone produces sufficient depression of automaticity in A to reduce it below B. Both influences combined, however, result in a shift in the seat of impulse formation. No shortening of the As-Vs interval occurs with the cooling or vagus stimulation alone, but is present when the two are combined.

A third possibility is concerned with the influence of somewhat greater depression produced by the cooling and stronger vagus stimulation. It may be represented diagrammatically as follows:

	A	*B*
Normal discharge	100	87
Vagus stimulation alone	75 ——→	→80
Cooling of A	85 ——→	→87
Cooling combined with vagus stimulation	75 ——→	→80

In this case, vagus stimulation alone, cooling alone and cooling combined with stimulation all cause a shift in the seat of impulse initiation. Cooling combined with vagus stimulation produces approximately the same degree of slowing as vagus stimulation before the cooling. Cooling alone involves a shortening of the As-Vs interval. The second series of Experiment 5, quoted in the preceding section will serve as an example illustrative of this type.

Finally, a fourth case occurs which is characterized by the fact that the vagus undergoes, as a result of the cooling, a relative as well as absolute diminution in its chronotropic influence over the heart. The effect of cooling combined with vagus stimulation produces less effect than vagus stimulation alone. This involves a stronger degree of depression by cooling and a stronger vagus stimulation and there is a double shift in the seat of impulse formation.

	A	*B*
Normal discharge	100	87
Vagus stimulation alone	70	68
Cooling of A	75 ——→	→87
Cooling combined with vagus stimulation	74 ←——	—68

Strong cooling of A causes its automaticity to be lowered to a point below that of B and the latter assumes the function of impulse initiation at a slower rate and with a shortened As-Vs

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interval. If now the vagus is stimulated, due to the fact that the cooling of A has to a great extent or entirely paralyzed the influence of the vagus on this region, B becomes depressed to a point below A and the seat of impulse initiation returns temporarily to A. The As-Vs interval lengthens during this time to that present normally. With the cessation of the vagus stimulation, the depression of B passes off, the function of impulses initiation returns to B and the As-Vs interval again becomes less than normal. As noted in the previous section, this result was obtained in eight of the twenty-five series involving cooling of the upper end of the sino-auricular node and subsequent stimulation of either the right or left vagus. An example is given by the second series quoted in the preceding section. In each case it was associated with periods of prolonged cooling with ice or intense cooling with ethyl-chloride and the employment of vagus stimulation of sufficient strength to produce a marked chronotropic effect in the normal heart.

The above diagrammatic representation of the possible changes in the rate of discharge in a single region or shifts in the location of the seat of discharge under the local depressing influences of cold and vagus stimulation, would seem at least to explain satisfactorily the various types that we have obtained experimentally. They accord in all details with the hypothesis of vagus action that we have adopted and we believe offer support to this hypothesis.

When the influence of the cold is not confined to a portion of the sino-auricular node, but is allowed to affect the whole node, the results that might be expected are capable of analysis, likewise, by the above method. The region A is to be regarded as the whole node rather than the upper part as in the previous discussion. Recent work would seem to demonstrate that if the sino-auricular node is removed from the rôle of pacemaker the seat of impulse initiation shifts, in the great majority of cases at least, to some part of the auriculo-ventricular node.[15]

[15] Cf. Ganter and Zahn: loc cit.; Zahn: Archiv f. d. gesamt. Physiol, 1913, cli, 247; Meek and Eyster: loc. cit., and Koch: Archiv f. d. gesamt. Physiol, 1913, cli, 279.

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This shift is clearly indicated by a lengthening of the cardiac cycle and by a marked shortening of the As-Vs interval, which frequently becomes zero under these circumstances. Due to this marked effect on the As-Vs interval the change in the seat of the pacemaker is readily followed experimentally. Rothberger and Winterberg[16] were the first to make the interesting observation that vagus stimulation during auriculo-ventricular rhythm may abolish this rhythm, as indicated by a lengthening of the As-Vs interval during the stimulation, to return to zero or at least become much shorter after the stimulation period is over. In a previous paper of the present series[17] it was demonstrated by galvanometric methods that under these conditions the seat of impulse formation returned to the sino-auricular node. In the present work, in every case but one, seven trials with the right and four with the left vagus, there was a return of sino-auricular rhythm as indicated by the lengthening of the As-Vs interval during the stimulation. In the single exception, the left vagus had no effect whatever on the auriculo-ventricular rhythm. Theoretically, it is evident that the production of an auriculo-ventricular rhythm by cooling the sinus node may lead when combined with vagus stimulation either to a greater or less effect on the normal rate than vagus stimulation before the cooling. The efficiency of the vagus is, however, in all cases decreased. This is evident from the following diagram:

	A(s–a node)	*B(a–v node)*	*A(s–a node)*	*B(a–v node)*
Normal discharge	100	80	100	80
Vagus stimulation alone	75	68	60	58
Cooling alone	75 ⟶	80	70 ⟶	80
Vagus stimulation combined with cooling.	70 ⟵	68	70 ⟵	58

These two types are illustrated by two examples in the previous section describing the effect of cooling the whole of the sino-auricular node. It is evident that the result in all cases involves a double shift in the location of the pacemaker, the first under the influence of the cooling, the second under the combined influence of the cooling and the vagus stimulation.

[16] Loc. cit.
[17] Loc. cit.

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We have made the assumption throughout this discussion that local cooling actually tends to reduce the chronotropic effect of the vagus supplied to the immediate region subjected to the cooling. For this we believe we have abundant experimental evidence. In those cases of mild cooling with relatively weak vagus stimulation, neither of which alone or combined are sufficient to cause a shift in the location of the pacemaker, there is always a somewhat diminished effect of the vagus. Furthermore, there are two instances in the present series of experiments which point strongly to this conclusion. In two cases, the left vagus lost all chronotropic influence upon the heart, in one as a result of local cooling of the upper part, in the other as a result of cooling the whole of the sino-auricular node. The only interpretation possible would seem to be that in these two animals the chronotropic fibers of the left vagus passed only to the upper part and whole of the node respectively and that the effect of the local cooling was to reduce their efficiency to zero. If there had been chronotropic fibers in these cases to other parts of the heart it would have been expected that by depressing the automaticity in these regions a return of the seat of impulse formation to the sino-auricular node would have resulted.

The results from the experiments in which the upper part, the remainder of the sino-auricular node and the auriculo-ventricular node were successively excised in the heart *in situ* under artificial perfusion, offer confirmatory evidence in support of the view that the chronotropic influence of the vagi is exerted on these structures or the closely neighboring regions and that this influence is progressively less in each region in the order named above. Removal of these structures in this order caused a progressive diminution in most cases of the chronotropic influence of each vagus. After complete removal of both nodes there was complete abolition of vagus influence in three of four experiments. In one a slight influence remained on the ventricle with strong stimulation.

The results which we have obtained we feel justify the following conclusions in reference to the chronotropic vagus control of the heart.

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1. *Both the right and left vagus normally exert their greatest chronotropic influence on the upper part of the sino-auricular node.* Removal of this region from the sphere of action by localized cooling, lessened the chronotropic influence of both vagi in each of four experiments. That such local cooling actually results, if sufficiently pronounced, in a temporary reduction of automaticity below the point of dominance we conclude from the associated lengthening of the cycle and shortening of the As-Vs interval, criteria which together are characteristic of a change in the seat of impulse formation as we have previously shown. The results from the experiments in which the upper part of the node was excised point strongly to the same conclusion as to the primal importance of this region for the chrontropic vagus influence.

In reference to the well recognized difference in action of the right and of the left vagus on the heart, it would seem that the usually greater chronotropic influence of the right vagus under normal condition is an expression of the fact that its supply of chronotropic fibers to the sino-auricular node is usually greater than that of the left. Our results point clearly to the conclusion, however, that the left vagus, as well as the right has normally a greater chronotropic influence over the sino-auricular than on the auriculo-ventricular node. In two of our experiments, as has been stated, the left vagus exerted no chronotropic influence on the latter region. Dromotropic effects on the auriculo-ventricular node, which seems to be usually the predominant influence of the left vagus over the heart, are to be sharply distinguished from the chronotropic influence. The left vagus may indeed cause a greater slowing of the ventricle than the right vagus, due to its chronotropic influence on the auricular rate combined with the production of a partial auriculo-ventricular heart block, while in the same animal the right vagus produces a greater chronotropic influence on the auricle than does the left vagus. The auricular rate is evidently the proper criterion for the chronotropic influence of either vagus.

2. *Both the right and the left vagus exert a greater chronotropic influence on the whole of the sino-auricular node than on regions*

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of lower automaticity. Cooling the whole node reduced the influence of both vagi in the three experiments in which this procedure was attempted. Excision of the whole node caused a similar reduction in the influence of both vagi in each of five experiments.[18]

3. *Both vagi usually supply the auriculo-ventricular node with chronotropic fibers.* This is well shown by the abolition of an auriculo-ventricular rhythm, resulting from cooling the sino-auricular node, by stimulation of the vagi. In two of the experiments the chronotropic influence of the left vagus was abolished after removal of the sino-auricular node as pacemaker. There were no similar instances of failure of the right vagus to supply this region with chronotropic fibers.

4. *The chronotropic influence of the vagi on the heart is mediated through the sino-auricular and auriculo-ventricular nodes, or at least within their immediate neighborhood.* Although great care was taken to localize the cooling and excision, it is obviously impossible to avoid some influence on the immediately surrounding tissues. The characteristic anatomical structure of these regions, the localization of effects produced in them or in the immediate neighborhood, as well as the previous work by galvanometric methods in associating these parts of the heart with that concerned under all normal conditions with impulse initiation, would seem to point strongly to this conclusion.

[18] On the basis of the well known fact that vagus stimulation may cause the abolition of auriculo-ventricular rhythm with return of the pacemaker to the sino-auricular node under conditions in which this structure is still capable of initiating impulses, Lewis in a recent paper (Heart, v, 247, 1914) concludes that the normal influence of the vagi is greater on the auriculo-ventricular than on the sino-auricular node. This assumption is clearly disproven, not merely by the fact that the efficiency of the vagi is reduced under these circumstances, but most clearly when such return of the seat of impulse formation to the sino-auricular node is prevented by its excision. Under these circumstances the relative as well as absolute lessened chronotropic influence of the vagi on the auriculo-ventricular node is clearly shown. The apparently greater influence of the vagi on this structure when the sino-auricular node is still intact is readily understandable if we recognize that the factor (cold) which depresses the automaticity of the sino-auricular node and which is thus the original cause of the auriculo-ventricular rhythm, also depresses the influences of the vagus on the sino-auricular node.

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5. *The mechanism of chronotropic vagus action is a local depression of automaticity in that region which is acting as the seat of impulse initiation.* This may involve a continuance of this region as pacemaker but a discharge at a slower rate, or it may involve a removal of the seat of impulse initiation to some region of normally lower automaticity, which, due to the lessened vagus influence on this region, is not depressed in equal proportion.

SUMMARY

The work reported in this paper represents an attempt to determine the relation of the sino-auricular and auriculo-ventricular nodes to the chronotropic function of the vagus nerves. Two groups of experiments were performed. In one a part or the whole of the sino-auricular node of the dog's heart was subjected to cooling and the effect of this studied on the chronotropic action of the vagus nerves. In the other, successive removal of the nodal tissues was carried out in the dog's heart beating *in situ* under artificial perfusion, and the influence of this procedure determined. It was found that local cooling of the upper part of the sino-auricular node, the region which recent work has shown to be the normal seat of impulse initiation, reduces the efficiency of the vagi. The cooling, by depressing the automaticity of this region has itself a negative chronotropic influence, and subsequent stimulation of the vagus may produce a greater diminution of the normal rate than stimulation with the same strength of current before cooling the node. The influence of the vagus on the rate after cooling is however less than its influence on the normal rate; the efficiency of the vagus is thus diminished. More intense cooling, or cooling for a longer period, was shown to so far decrease the efficiency of the vagus that the cooling combined with vagus stimulation produced less effect on the normal rate than stimulation of the vagus with the same strength of current alone. It was shown how on theoretical grounds these changes could be explained by a change in the seat of impulse initiation to regions of lower automaticity and application of the experimental facts was made

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to those theoretical interpretations. Cooling the whole of the sino-auricular node produced auriculo-ventricular rhythm, abolished on subsequent vagus stimulation. Interpretation of the change in this case was compared with that in the preceding cases. Successive removal of the nodal tissue from the heart tended to show the close association of these structures with the chronotropic function of the vagus and to lend support to the view that the control of the vagus over the automaticity of this tissue decreases progressively from above downward. The work is offered as lending confirmatory evidence to the hypothesis of chronotropic vagus action previously put forward, namely, that the vagus produces its chronotropic effect on the heart by local depression of automaticity in the nodal tissues, causing the seat of impulse initiation to undergo progressive shifting to regions of lower automaticity.

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AXIAL GRADIENTS IN THE EARLY DEVELOPMENT OF THE STARFISH

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The data presented here were obtained during the summer of 1913 and 1914 at the Marine Biological Laboratory at Woods Hole. Attention has been called in earlier papers to the existence of these axial metabolic gradients in animals and to their significance in individuation and development.[1] My earlier observations were mostly upon adult forms or later stages of development, but within the last two years the embryonic stages of a number of species have been examined and the present paper gives the results for the starfish (*Asterias forbesii*).

METHODS

The method chiefly employed to demonstrate the gradient was the susceptibility method which I have discussed elsewhere (Child, '13 a), with KCN, in most cases 0.01 *m.*, as reagent, and both the disintegrative changes and the limits of recovery were determined in many cases. In the first case the eggs or embryos were placed in the KCN solution in sea-water and the course of death and disintegration along the axis was observed microscopically without removal from the solution; in the second place portions of the material were removed from the KCN at regular intervals, washed in several changes of sea-water and the limits of recovery of different regions in sea-water determined by further observation. The results of these two procedures agree in all cases.

[1] See Child, '11 a, '11 b, '11 c, '11 d, '12, '13 b, '13 c, '14 a, '14 b, '14 c, '14 d.

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The method used by R. S. Lillie ('01, '13) for determining the localization of intracellular oxidations was also used for the starfish with very interesting results. This method depends upon the intracellular oxidation of a mixture of dimethyl-*p*-phenylene-diamine, $C_6H_4 \cdot NH_2 \cdot N(CH_3)_2$, and α-naphthol, giving rise to dimethyl-indophenol, a deep blue compound, which is deposited in granular form in the cells. Lillie employed the substances in molecular concentration in 50 per cent alcohol and only for dead tissues and cells: in order to make the method available for living forms it was necessary to use much lower concentrations with sea-water instead of alcohol as a solvent. Since the physical condition of the dimethyl-*p*-phenylene-diamine made exact weighing difficult and since it was found that exact proportions of the two substances were entirely unnecessary, I worked with very dilute solutions made up as follows: To 4 or 5 cc. of a solution in sea-water of the diamine ranging from 1–5 per cent (estimated) two to five drops of a saturated or nearly saturated solution of α-naphthol in sea-water were added, this mixture was made slightly alkaline and five to twenty drops of it were added to several cubic centimeters of sea-water containing the eggs or embryos in a Syracuse glass. In such solutions swimming starfish larvae live from 10 minutes to an hour at room temperature and during this time the differential staining along the axis by the formation of indophenol becomes very distinct. Since the staining occurs even in very dilute solutions the chief precaution necessary is sufficient dilution, particularly of the α-naphthol, which is much more toxic than the diamine, so that the animals may be kept alive as long as possible.

THE POLAR GRADIENT IN SUSCEPTIBILITY

The unfertilized egg

In these stages the presence of a gradient in susceptibility was determined by the progress of the disintegrative changes over the egg in KCN. During growth the ovarian eggs remain connected with the parent body by a short stalk, but I have

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not been able to distinguish with certainty any susceptibility gradient in relation to the point of attachment as a characteristic feature of these stages. In some of the younger eggs disintegration does proceed from the point of attachment but in many others no gradient is visible.

As the egg grows and the cytoplasm increases the nucleus becomes eccentric in position, but its eccentricity apparently has no relation to the point of attachment. Possibly this eccentricity of the nucleus may be related to the oxygen supply at the surface of the egg: the nucleus may approach the surface where a space between the closely packed eggs is present. But whatever the factor which determines the nuclear position, Wilson and Mathews ('95) have shown that that region of the egg where the nucleus lies nearest the surface becomes the apical or animal pole. When maturation begins, the asters make their first appearance at that part of the nuclear periphery which is nearest the egg surface and the disappearance of the nuclear membrane begins here. In the full grown starfish egg maturation begins at once without fertilization when the egg is removed from the ovary to water. Evidently this is the region of greatest activity in the egg or where activity increases most rapidly when the egg is removed from the ovary to water.

When full grown eggs are brought directly from the ovary into KCN 0.01 *m.* the germinal vesicle disappears in many of them as it does in water, while others remain unchanged, the proportions varying with different lots of eggs. In the eggs which remain unchanged a gradient in susceptibility to cyanide usually appears in the course of disintegration. The disintegrative change consists in the breakdown of the cytoplasm into droplets and granules accompanied by swelling until finally the structure of the living egg has completely disappeared. This change usually begins in that region of the egg periphery where the nucleus is nearest the surface and progresses through the egg, the line of demarcation between intact and disintegrated portions being distinct and the intact portions retaining their form.

In many of these eggs the germinal vesicle is completely ex-

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truded at that point of the egg surface nearest to it before disintegration of the cytoplasm begins, and the cytoplasm may remain intact for some time after its extrusion: when disintegration finally occurs in such eggs it is often impossible to determine whether the gradient corresponds with the axis determined by the nuclear position. In some cases, however, the extrusion of the nucleus does not occur until after cytoplasmic disintegration has begun and in such eggs the disintegration gradient does coincide with the axis. Figure 1 shows the beginning of disintegration in such an egg, the disintegrated cytoplasm being diagrammatically indicated.

The extruded nuclei often remain intact and normal in appearance for some hours, but when they finally disintegrate they show in most cases a very striking gradient. As in the cytoplasm, the disintegration begins at one point of the nuclear surface and proceeds through the nucleus in a definite direction with a sharp line of demarcation between intact and disintegrated portions. Figure 2 shows four stages in the nuclear disintegration. At the time when it disintegrates the nucleus is evidently a rather firm gel and disintegration of part of the membrane or part of the nuclear contents does not alter the form of the rest. In these isolated nuclei it is of course impossible to determine whether this gradient coincided in direction with the cytoplasmic gradient, but it is difficult to account for its existence except in relation to the cytoplasm and the eccentric position of the nucleus. In all probability nuclear and cytoplasmic gradients coincide in direction and result from the same conditions.

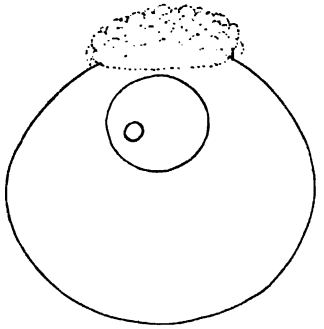


Fig. 1

In those eggs in which the germinal vesicle breaks down after they are placed in cyanide, or in eggs which are left in water for a few moments after removal from the ovary to permit maturation to begin and are then placed in cyanide, the cytoplasmic gradient in susceptibility is more marked. The region

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where the polar spindle is forming or has formed is the most susceptible and disintegrates first and from this region disintegration proceeds through the egg along a definite axis. Figure 3 represents the beginning of disintegration in an egg at this stage, the region where the polar spindle is forming being indicated diagrammatically; Figure 4 shows a more advanced stage of disintegration. These eggs are also somewhat more susceptible and disintegrate earlier than those with intact germinal vesicle, and this increase in susceptibility is undoubtedly associated

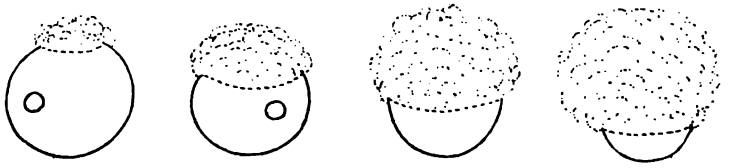


Fig. 2

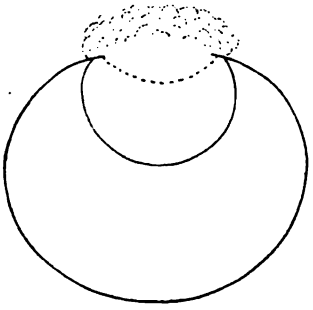


Fig. 3

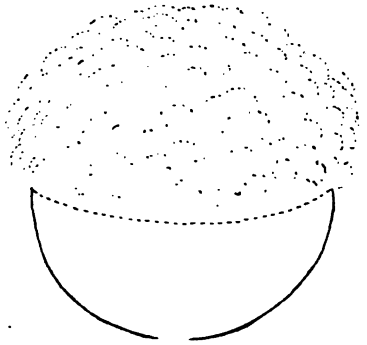


Fig. 4

with the increase in metabolic activity which occurs as maturation begins.

Summing up, these observations on the unfertilized egg indicate that a gradient in susceptibility to cyanide occurs in both cytoplasm and nucleus, in the direction of the axis determined by the eccentric position of the nucleus and that this gradient becomes more distinct as the metabolic activity in the egg increases with the beginning of maturation. The region of highest susceptibility in this gradient becomes the animal pole of the egg and the apical region of the larva.

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The earlier cleavage stages

The earlier cleavages in the starfish are so nearly equal that it is impossible to determine the position of the animal pole except by the polar bodies and it is not by any means certain that these always retain their original position; moreover, if disintegration begins at the animal pole, the polar bodies are either lost at once in the disintegrated cytoplasm or themselves disintegrate so that no landmark remains. Periodic changes in susceptibility connected with division which were first observed by Lyon ('02, '04) in the sea urchin egg may also complicate the situation, for certain blastomeres may be somewhat in advance of or behind others and so be more or less susceptible at a given time. For these reasons it is usually impossible to be certain that susceptibility gradients observed during the early cleavage stages coincide with the polar axis of the egg.

In the two-cell and four-cell stages disintegration gradients often appear in each blastomere and in such cases there is every reason to believe that they coincide with the egg axis. In many cases, however, one or two blastomeres are more susceptible than the other or others and disintegrate earlier, doubtless because the periodic changes in susceptibility do not coincide in time.

As cleavage continues, the susceptibility gradient, as shown by the course of disintegration becomes again more distinct and we find that disintegration usually begins in one region of the embryo and proceeds from this region over the whole. Even here, however, irregularities occur and blastomeres or blastomere groups sometimes disintegrate before the disintegration gradient has reached them. Here again it is probable that such cells are in certain stages of the division cycle in which they are more susceptible that the cells surrounding them.

In short, evidence for the existence of susceptibility gradients during these stages is not lacking, but the difficulty lies in identifying the axes of these gradients with the egg axis. Nevertheless, the observations on the unfertilized egg and on later developmental stages constitute adequate grounds for believing that

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the axial gradient does exist during the early cleavage and that the susceptibility gradients observed do coincide with the axis, except where incidental factors temporarily mask the fundamental gradient.

The blastula

In these stages there is no question concerning the presence and definiteness of the susceptibility gradient. It can be demonstrated either by the course of death and disintegration or by the limits of recovery. In the early spherical blastula before movement begins the disintegration gradient is distinct, but the difficulty in identifying the animal pole and embryonic axis makes it impossible to demonstrate that this gradient coincides with the axis. In later free swimming stages of the blastula the direction of movement with the apical region, the animal pole, in advance and before gastrulation the elongation of the embryo in the direction of the axis and the increasing thickness of the cellular layer toward the vegetative pole render orientation of the embryo possible at a glance. In these stages the disintegration gradient is very distinct and without noteworthy variation. Disintegration begins at the apical end and proceeds with a definite course along the embryonic axis, ending in the region of the vegetative pole where the gastrular invagination will occur. Death and disintegration of the apical region of the embryo begin while the animal is still swimming about and movement may continue until the apical half of the body is disintegrated. The disintegrative process in this stage consists first of the rounding and separation of the cells and second of their complete disintegration. In many cases the progressive loss of cells beginning at the apical region can be seen as the blastulae swim about. As disintegration proceeds down the axis movement becomes less and less rapid and finally ceases, although the cilia of the cells in the region of the vegetative pole may continue to beat after locomotion has ceased.

The existence of the axial gradient can be demonstrated not only directly by observing the course of death and disintegration but indirectly as well by removing the animals to water and so

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stopping death at any level of the body and allowing recovery to occur. The use of recovery in the earlier stages of development as a means of demonstrating the gradient is open to the objection that regulatory reconstitution of the part remaining alive into a dwarf whole may occur and so make conclusions uncertain. This objection does not apply to the late blastula stages for gastrulation occurs almost at once after recovery and before any extensive regulatory changes take place.

The same gradient appears in recovery as in the course of disintegration. By removing the animals to water at proper intervals it is possible to stop death at any level of the body from the apical end downward. In such cases the apical end of the living portion closes and in the larger partial larvae thus obtained gastrulation occurs.

In these stages then the results are perfectly definite and beyond all question and when we compare them with the results in the egg undergoing maturation there is little possibility of doubt that the susceptibility gradient observed during cleavage coincides with the axis of the egg and embryo.

The gastrula

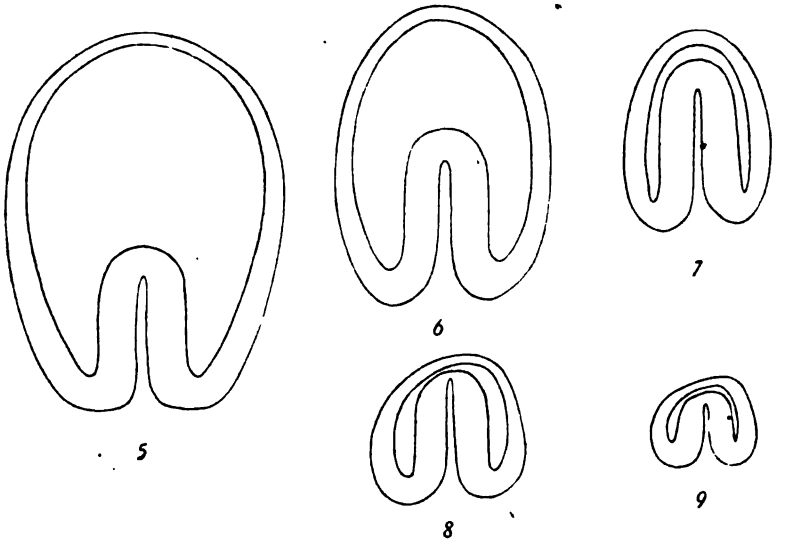
The susceptibility gradient of the gastrula is similar to that of the blastula, the susceptibility being greatest in the apical region and least in the region of the blastopore, with a gradation in the intermediate regions. Some idea of the steepness of the gradient, i.e. of the differences in susceptibility along the axis may be gained from the fact that the average survival time of early gastrulae in KCN 0.01 *m.* at a temperature varying from 20–23 C. is about one and one-half hours and the length of time between death of the first apical cells and death of the blastopore region half an hour to an hour.

The gradient in the wall of the archenteron is similar to that in the body wall, the apical end of the entodermal invagination being the region of greatest susceptibility. As in the blastula stage, the gradient can be directly demonstrated by the course of death and disintegration from the apical pole to the blasto-

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pore or indirectly by allowing recovery at different intervals. At the one extreme of recovery death may be limited to a few apical cells, at the other only the blastopore region remains alive and between these two extremes the stopping of death at any desired level of the body is merely a matter of removal from the cyanide to water at the proper time.

The partial gastrulae obtained in this way are of some interest and a few examples are shown in figures 5–9. Figure 5 represents a normal gastrula; figure 6 is a partial form in which death



Figs. 5-9

has proceeded from the apical region over about one-third of the body-length and then recovery and closure of the open apical end has occurred; figure 7 is a case in which the apical half or more of the body-wall died; in figure 8 some three-fourths of the body-wall and the tip of the entodermal invagination failed to recover, and in figure 9 only the extreme basal region of the body-wall and the stump of the entoderm have recovered.

In most of these partial gastrulae a considerable portion of the dying disintegrating cells pass into the blastocoel instead of being cast off externally so that in the partial larvae after recovery

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the blastocoel is usually more or less completely filled with cellular débris, which gradually disappears, evidently serving as a source of nutrition for these larvae usually live longer than those without such débris. Where the part which recovers consists of the basal half or less of the body the blastocoel is much reduced in size as in figures 7–9 because that part of the ectoderm which remains intact is barely sufficient to enclose the entodermal invagination.

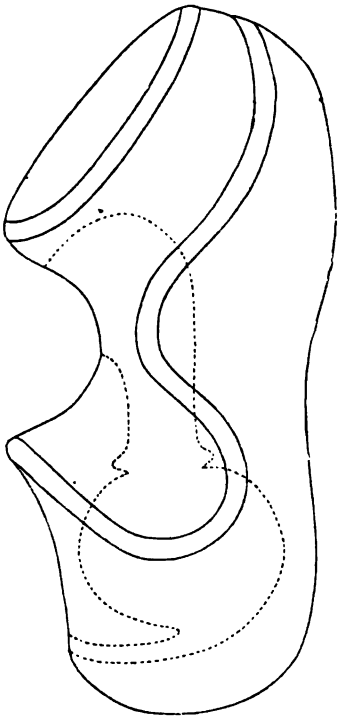


Fig. 10

These partial gastrulae, particularly the larger ones, undergo regulatory changes and often develop into larvae of normal form and structure but dwarfed to a greater or less degree according as they represent larger or smaller portions of the original body. In the smallest partial gastrulae representing only the basal end of the original gastrula some enlargement of the blastocoel and elongation of the entodermal stump may occur before death, but development has not proceeded farther than this in any case observed.

Later stages

The chief feature of the development of the bipinnaria larva from the gastrula are increase in size, formation of the mouth and stomodeum, development of the ciliated bands and the accompanying changes of shape (cf. figs. 5 and 10). During this period the axial susceptibility gradient becomes less and less distinct until in the stage of figure 10 it is in most individuals scarcely appreciable and sooner or later disappears and in many individuals the posterior region of the body shows a slightly higher susceptibility than the anterior, at least as regards the ectoderm.

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These changes are due to the fact that the anterior (apical) regions of the body which were originally the regions of highest metabolic rate have developed more rapidly than the posterior region and therefore have attained their maximum rate of reaction and have begun to undergo decrease in rate before the posterior regions attain their maximum. Consequently the original susceptibility gradient which results from the differences in metabolic activity along the axis first disappears in these later stages and is then replaced by a slight gradient in the reverse direction. In the latest larval stages examined no constant axial gradient could be observed with certainty.

Later stages of development have not been obtained and nothing is known as yet concerning the origin of new gradients during metamorphosis.

THE SYMMETRY GRADIENTS IN SUSCEPTIBILITY

The starfish larva is bilaterally symmetrical and the first morphological indications of this symmetry are those concerned in the formation of the mouth, the ingrowth of the stomodeum and its union with the entoderm. The gastrula before this stage is to all appearances radially symmetrical (fig. 5).

In view of the fact that a susceptibility gradient exists along the polar axis of the body the question whether similar gradients exist in relation to the planes or axes of symmetry becomes of considerable importance. It is difficult, however, to obtain conclusive evidence upon this point. In the stages before bilateral symmetry becomes morphologically visible, even after the polar axis is readily distinguishable, there is no way of determining which will become oral and which aboral surface and it is therefore impossible to determine with certainty whether differences in the susceptibility gradient along different meridians of the body have any relation to the later symmetry. As a matter of fact, observations on the later blastula stages where the body-form makes the identification of the polar axis easy and on the gastrula show that death and disintegration do proceed more rapidly along one side of the body than along the

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others. In one series particularly, in which blastulae just before movement were placed in the cyanide solution and removed at intervals for recovery, the oral wall of the body showed itself to be more susceptible than either the aboral or lateral regions. The portions which recovered developed into larvae with apical and oral defects varying in extent with the proportion of the body killed. Those which underwent recovery in the early stages of death showed only apical loss of cells, but after a longer time in the cyanide the apical defect was more extensive and the defective condition extended down the oral side of the body, in the more extreme cases even to the blastopore. The radial asymmetry evident in many partial gastrulae (see figs. 8 and 9) results from the presence of the symmetry gradients, one side of the body—presumably the oral—being somewhat more susceptible.

These facts indicate that the median region on the oral side of the body possesses a higher susceptibility than either lateral or aboral regions; i.e., that a downward gradient in susceptibility extends laterally and aborally around the body in both directions from the median oral region. If this is true, the axes or planes of symmetry of the larva as well as the apico-basal or polar axis are represented by susceptibility gradients.

The oral-lateral-aboral gradient, like the polar gradient, becomes indistinguishable in the bipinnaria stage when development comes to a standstill in the absence of proper conditions for metamorphosis.

DEMONSTRATION OF THE POLAR GRADIENT BY THE INDOPHENOL REACTION

As an indicator of gradients in metabolic condition the indophenol reaction is less delicate than the differential susceptibility to cyanide. In the stages preceding fertilization the results with the indophenol reaction are less definite and positive than those with the cyanide, but, so far as they go, confirm the latter. In the earlier cleavage stages much the same is true, but in the blastula and gastrula the staining gradient becomes

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very clearly marked. Staining always begins at the apical pole and spreads gradually in the basal direction while the animals are still swimming about. The apical third or half of the body is stained a deep blue before the blastopore region has become stained at all.

If the animals live long enough, the color gradient disappears by the gradual extension over the whole body of the deep blue color, which appears first in the apical region and death and disintegration very soon follow this condition. Moreover, animals which have been killed in any other way before being placed in the staining solution, or which are killed at once by a too highly concentrated solution, stain uniformly throughout without any indications of a gradient at any time. Evidently the staining gradient, like the susceptibility gradient, exists only in the living, active animal.

As regards the symmetry gradients, I believe that I have observed more rapid extension basally of the staining along a certain meridian of the body in gastrulae, but it is impossible to demonstrate that this is the oral side of the body. In some cases after the mouth has appeared the region of the oral meridian seems to stain slightly earlier than others but the difference is not very great and disappears later.

This method of demonstrating the gradient is of value chiefly as a confirmation of the susceptibility method and it is of interest to find the two methods are in agreement as far as they give positive results.

DISCUSSION

As regards the nature of the gradient in susceptibility to cyanide, various lines of evidence (Child, '13 a, '13 c) show very clearly that susceptibility to cyanide in concentrations which are lethal in the course of a few hours varies with the general rate of metabolic activity, or of certain fundamental metabolic reactions, perhaps the oxidations. And this conclusion holds whether the cyanide acts more or less directly upon the oxidations or upon the condition of the metabolic substratum or certain of its constituents and so indirectly upon metabolic

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activity in general. The susceptibility gradient as observed in the starfish is in short a gradient in rate of metabolic reaction in the organism and the region of highest susceptibility is the region of highest rate. The parallelism between susceptibility and metabolic activity holds only for the higher concentrations of cyanide. In concentrations low enough to permit some degree of acclimation the region of the body which is most susceptible to the higher concentrations becomes least susceptible because it becomes most readily and most completely acclimated. Under such conditions then the susceptibility gradient is reversed, although the metabolic gradient remains the same. This reversal of the susceptibility gradient with change in concentration demonstrates that the gradient does not depend primarily upon fixed structural conditions along the axis but rather upon the dynamic changes which are going on.

The gradient in the rate of the indophenol reaction indicates a gradient in rate of oxidation presumably in consequence of a gradient in the amount of activity of oxidizing enzymes or in other conditions which influence intracellular oxidations. This method simply confirms the susceptibility method in demonstrating that a quantitative metabolic gradient exists along the axis or axes of the body, at least during certain stages of development.

The conclusions which I have reached concerning the rôle which gradients play in the organism can be only briefly reviewed here. I have presented various lines of evidence which indicate that an organic axis in its simplest terms is a quantitative gradient. I have also pointed out that the orderly sequence in space and time of developmental events is related to the existence of a gradient or gradients. And finally, the region of highest rate in the gradient dominates or controls regions of lower rate within a certain distance. This relation of dominance and subordination depends primarily upon differences in metabolic rate. The metabolic changes or their effects spread, irradiate, or are transmitted and the influence of the region of highest rate upon other parts by transmission of this kind is manifestly greater than their influence upon it. Throughout the organic world, in both plants and animals, the region of

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highest rate of reaction in the chief axial gradient becomes the apical region or head of the organism and the final expression of the relation of dominance and subordination is the central nervous system. It is a fact of great importance that the central nervous system arises from the region of highest rate in each gradient: the cephalic portion of the nervous system develops from the region of highest rate in the whole organism, and in most of the bilaterally symmetrical invertebrates the median ventral, in the vertebrates the median dorsal region represents the region of highest rate in the symmetry gradients and along this line the longitudinal portion of the nervous system arises. In short the axial metabolic gradient is, I believe, the first step in the physiological integration of the axiate organic individual. The relations established by the gradient or gradients lead through the various phases of development to their final expression in animals of functional integration through the nervous system.

The present paper is merely one fragment of experimental evidence in support of these conclusions. It shows that the axial gradients exist during certain developmental stages. It is also of interest to note that the direction of the polar gradient is apparently determined by chance or incidental factors, for it arises in relation to the position of the nucleus which is apparently determined by such factors. It is not clear how the symmetry gradient is determined, though it may be that the entrance of the spermatozoon provides a starting point for metabolic differences in different egg-meridians, as is apparently the case in certain other forms.

The occurrence of agamic reproduction in consequence of physiological isolation of parts of the body from the controlling influence of the dominant region has been demonstrated experimentally for various forms and shown to be probable for many others (Child, '10, '11 a, '11 d). While there is no question of reproduction in the ordinary sense in the starfish larva, the disappearance of the primary gradients preceding metamorphosis is an interesting and, I believe, a significant fact, for it is probably this disappearance which makes possible the establishment

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of new gradients and so of new axes in relation to other factors and initiates the development of the starfish body in the larva. In the final analysis the metabolic gradient must always be determined by the action of incident factors external to the protoplasm, cell or cell mass concerned and in the starfish we should probably find, with adequate technique, that the metabolic relations of parts to each other and to external factors determine the origin of the new axes of the starfish body.

SUMMARY

1. A quantitative metabolic gradient, distinguishable both by differences in susceptibility to cyanides and by the rate of oxidative formation of indophenol in the cells is present in the unfertilized starfish egg, probably persists through the cleavage stages and is very distinct in the blastula and gastrula and finally disappears in the bipinnaria larva as metamorphosis approaches.

2. This gradient coincides in direction with the axis determined by the eccentric position of the nucleus in the ovarian egg.

3. The region of highest metabolic rate in this axial gradient becomes the animal pole of the egg and the apical region of the larva.

4. Somewhat less complete evidence indicates the existence of symmetry gradients in which the region of highest rate becomes the oral side of the larval body, the region of lowest rate the aboral side.

5. These metabolic gradients are directly related only to the larval axes. As the larva approaches metamorphosis they disappear and later the new axial gradients of the starfish body arise in the larva.

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AN ANALYSIS OF EXPERIMENTAL EDEMA IN FROGS

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In discussions regarding the nature of edema attention has frequently been called to the pathological condition obtained in a frog's leg by ligating it tightly about the knee. If a frog operated on in this way is kept in water, the result is an accumulation of lymph-like fluid below the ligature and a swelling of the muscle which leads finally to rigor. In general the same phenomena occur whether the leg be severed from the body or not. The imbibition and retention of the water by the muscle, Loeb has suggested, may be due to an increase in the osmotic pressure within the tissue because of chemical changes due to the lack of oxygen (1).

Various later writers basing their conclusion upon an observation of Loeb's viz.: that eventually an artificial edema takes on an acid reaction, regard edema in general as due to the production of acid within the tissue cells, and assume that lack of oxygen causes the formation of acid in sufficient abundance to cause the tissues to swell (2) i.e., imbibe water.

Now it has been shown that lactic acid must be present in Ringer's solution in relatively high concentration in order to cause the excised gastrocnemius of a frog when immersed in it to swell (3). Recently Henderson (4) has shown that a solution having the acidity of human urine, is not sufficient to cause a muscle to take up water when the muscle is kept in the solution for several hours. It is always to be noted in experiments on muscle swelling in acid solution, that before any significant increase in weight takes place, the muscle loses its irritability. That is to say, excessive water absorption is attended by a moribund condition of the tissue. In regard to this point,

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Meigs (5) has shown that frog's dead muscle behaves like a colloid since it swells in a sugar solution as in distilled water, but that live muscle acts like an osmotic system in that its weight is governed by the concentration of the surrounding solution independently of its chemical composition. Therefore, while admitting that acid acts upon dead muscle in exactly the same fashion as it acts upon a gelatin plate, we still lack proof that such facts necessarily have a bearing upon the problem of water absorption and retention by live muscle, and hence we may safely discard any theory which seeks to account for the absorption of abnormal quantities of water by muscle on the hypothesis of acid formation and accumulation within the cells. Furthermore such an hypothesis does not take into account one characteristic feature of edema, viz.: the accumulation of lymph-like fluid outside the muscle (6).

Since practically all the experimental support for the colloidal chemical view of edema depends upon an interpretation of the nature of experimental edemas in frogs' legs, it seems that the whole situation may be cleared if we can arrive at an intelligent conception of the causes underlying such an edema. It is our purpose, therefore, to determine just what factors are involved in the production of the experimental edemas in question. From what has been said it is evident that the problem divides itself into two parts:

First, What causes the accumulation of lymph-like liquid between the skin and muscle?

Second, Under what conditions does the muscle increase in weight and become hypotonic to normal serum? In other words, why does the muscle absorb and retain abnormal quantities of water?

As a preliminary step we should know what factors determine the normal water absorption of a frog immersed in water. Does the skin limit the amount of water taken in, or is the water content of the tissues and plasma kept normal by the action of the circulatory and excretory systems? The first part of the question has been answered by Maxwell (7) who showed by means of conductivity measurements that water passes through the

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frog's skin entirely in accordance with the laws of osmosis. The part played by the circulatory and excretory systems has been made clear by Overton (8) who showed that if the cloaca of a frog be ligatured and the animal kept in pure water, its body weight increases, the bladder becomes filled with dilute urine and finally the intestine itself is flooded. If, however, a frog with a closed cloaca be put into $\frac{M}{6}$ NaCl solution no change in weight takes place, that is to say, water is neither gained nor lost by the animal since the fluid in which it is kept is isosmotic with the frog's plasma and tissues. The facts which Overton and Maxwell have established point to the conclusion that there is a constant flow of water through the skin toward the tissues in the case of a frog immersed in water and that the excess water is removed by way of the kidneys and bladder.

Thus, after the water has passed through the skin it is carried away in the lymph or blood, by the lymphatics or veins or both. Tightly ligating the knee of a frog would lead to an accumulation of fluid composed of transudate and absorbed water below the ligature, simply by stopping the drainage. Volhard (9) has concluded that the lymphatics alone are responsible for the accumulation of liquid below a ligature. He closed the lymphatics of the lower leg of the frog by passing a ligature about the leg, under the veins and arteries just above the knee, and obtained an accumulation of lymph in the lower leg although the blood circulation continued normally. He also showed that ligating the arteries alone did not lead to lymph accumulation.

The present writer has repeated and confirmed Volhard's experiments. Further it has been found possible to cause an abundant accumulation of lymph in the leg of the frog simply by tying off the superficial lymphatic at the knee. This can be done by making a short longitudinal slit through the skin just above the knee on the dorsal side, passing a ligature around the leg under the skin and tying the ligature on the ventral side so as to include only the skin. Such an operation we shall term a lymphatic ligature. It has the advantage over Volhard's method in that only the lymphatic vessel is cut off while the veins, arteries and muscles are left uninjured in their normal

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situations. As a rule within a few hours after the ligature has been made the whole lower leg becomes distended with lymph, the quantity in a bull frog often amounting to 3 or 4 ccm. in a single leg. Such an accumulation takes place even if the frog is not immersed in water, in which case the lymph is retained transudate. Experiments in which the veins alone were ligated above the knee led to slight lymph accumulation or none at all. Therefore, we must agree with Volhard, in the main, that the retention of the extramuscular fluid is due almost entirely to the blocking of the lymphatics.

There remains the possibility that the veins may assist in the removal of the superfluous water which is being imbibed through the skin. If the lymphatics were the sole agents in the removal of this water, then the lymph which accumulates below a lymphatic ligature should be dilute, there being no chance of water removal. The establishment of this fact would prove the lymphatics to be the only means by which liquid can be removed from a limb.

Since the refractive index of a solution varies directly with the amount of dissolved substances contained, we have at hand an easy method of measuring the concentration of small quantities of lymph (10), (11). By the method of measuring the refractive index it was attempted to determine the differences in the concentration of dissolved substances in normal lymph and in that obtained from experimental edemas. In our experiments the Abbe type of refractometer was used. With this instrument a drop of liquid is sufficient to give an accurate reading. Table I shows a number of determinations of the refractive index of frog's lymph. The first column gives the refractive indices of samples of lymph from lymphatic ligatures, while opposite in the second column are the refractive indices of normal lymph. Each pair of measurements is made from samples of lymph from the same frog, the normal lymph always being obtained from the opposite unoperated leg. Comparable measurements were made at the same temperature, usually 20°.[1]

[1] Experimental error ± 0.0002.

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TABLE I

	LYMPH FROM LYMPHATIC LIGATURE	LYMPH FROM NORMAL LEG	DIFFERENCE
A.....	1.3372	1.3372	0.0000
B.....	1.3365	1.3370	0.0005
C.....	1.3380	1.3380	0.0000
D.....	1.3365	1.3366	0.0001

A large number of observations showed that while the variations in the concentration of dissolved substances in normal lymph were considerable in comparing different animals, samples from the two legs of the same animal as a rule gave identical readings. Table I shows that in spite of the ligature of the lymphatics, the lymph from an experimental edema does not vary significantly from normal lymph in its concentration. This means that in the edema, the lymph does not retain an excess of absorbed water. If water is taken up in an experimental edema made by a lymphatic ligature it must be removed by channels other than the lymphatics.

It can be shown that water is absorbed by the lymph in the case of experimental edemas in the following way. If we close every avenue through which the water may be removed by tightly ligating the leg at the knee, then severing it from the body above the ligature and afterward keeping the preparation in water for a number of hours, it will be found that the extramuscular fluid suffers dilution as shown by lowering of the refractive index. Such an experiment with the legs of two frogs is given in Table II. One of the preparations was opened after having been ten hours in distilled water, the others were opened at the end of twenty hours, and the refractive index of the extramuscular fluid determined.

TABLE II

	NO. I	DIFFERENCE	NO. II	DIFFERENCE
Refractive index normal lymph.....	1.3380		1.3378	
Refractive index 10 hours....	1.3365	0.0015		
Refractive index 20 hours....	1.3351	0.0029	1.3350	0.0028

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Other experiments carried out in the same fashion at other times showed differences in the refractive index of the lymph in the same sense. This means that the lymph becomes more and more dilute as water is absorbed by a limb having its circulation entirely interrupted. The muscles in such preparations likewise take up and retain excess water as is indicated by the fact that the gastrocnemii lose weight when excised and put into $\frac{M}{8}$ NaCl solution.

It is evident then that when the leg is completely ligated, and the blood vessels and lymphatics both effectually cut off, absorbed water must be retained. Our results show that in such an experiment both lymph and tissues contain excess water and are hypotonic. It becomes of interest to determine the mechanism by which the lymph in the case of the lymphatic ligature is kept at normal concentration as shown in Table I. Two possibilities are open. The muscle cells may take up water and retain it, or they may, after absorbing it, pass it to the capillaries and thence to the veins. By the conditions of the experiment the lymphatics are excluded. The elucidation of this problem will answer our second question, "What factors in an experimental edema cause a muscle to increase in weight and to become hypotonic, i.e., retain water?"

Let us first see to what extent muscle cells take up water under such circumstances. The water absorption by a muscle in an experimental edema produced by a lymphatic ligature is marked but not great. This is indicated by the following table. In obtaining these measurements the gastrocnemius muscles were removed at the same time from normal and ligated sides, then put into beakers containing Ringer's solution isotonic with frog's blood. From time to time the muscles were taken from

TABLE III

HOURS IN RINGER'S SOLUTION	LIGATED SIDE WEIGHT IN MGM.	PER CENT LOSS	NORMAL SIDE WEIGHT IN MGM.	PER CENT LOSS
0	260		235	
2½	245	5.8	235	0.0
5	240	7.7	225	4.2
11	230	11.5	235	0.0

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the solution and weighed in the usual manner. The lymphatic ligature in this case had lasted nineteen hours during which time the frog was immersed in water.

The loss in weight sustained by the muscle from the ligated side is greater than any corresponding loss in weight suffered by the normal muscle. The experiment shows clearly that a lymphatic ligature alone results in a certain amount of water retention in the cells. The amount of water retained never exceeds rather low limits, however, for rigor never sets in and the muscles may function for a week in the living animal. How, then, is the hypotonicity kept at this low level? We have seen that there is a constant flow of water inward through the frog's skin, that this water does not serve to dilute the lymph appreciably, and that the muscle absorbs and retains a certain amount of the water. If the latter process were not limited the muscle would soon go into rigor and become functionless. The only possibility of water removal remaining is that such removal takes place through the capillaries and veins. In order to test this hypothesis the following experiment was performed (12).

The gastrocnemius muscle of a frog was exposed in situ by cutting away the skin, care being taken not to injure the muscle. The blood circulation was therefore left intact. In the course of from one to four hours, varying with the condition of the frog, the muscle became swollen and hypotonic, showing a loss of from 10 per cent to 15 per cent of its weight when removed and put into Ringer's solution. But an excised muscle kept in distilled water gains about 20 per cent in one and a half hours and about 60 per cent in four hours. Apparently the lower degree of hypotonicity of the muscle which remains in situ depends upon the fact that the circulation is intact. Therefore if we should, in addition to removing the skin from about the gastrocnemius also ligature the femoral vein, as regards drainage, the condition of the muscle would be comparable to that of an excised muscle. In such an experiment where the skin was removed from about the gastrocnemii and the femoral vein of the right

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leg ligated, the animal was kept in tap water one and one-half hours. At the end of that time it was killed and both gastrocnemii were removed and put into Ringer's solution. The muscle from the right side showed nearly double the hypotonicity of that from the left.

TABLE IV

HOURS IN RINGER'S SOLUTION	RIGHT SIDE WEIGHT IN MGM.	PER CENT LOSS	LEFT SIDE WEIGHT IN MGM.	PER CENT LOSS
0	280		225	
2	220	21.4	200	11.1
6	210	25.0	190	15.1

That is to say, where the venous channel was blocked on the right side, the water retention by the muscle was almost double that shown by the opposite side where the circulation was intact. This definitely fixes upon the veins the responsibility for maintaining the water content of muscle and lymph at a low level in experimental edemas produced by the lymphatic ligature alone. The experiment was varied by ligating the arteries of one side, the veins being left intact. The result was to make the veins less effective in removing water by eliminating the driving force in the capillaries.

This is shown in Table V. The skins were removed from about both gastrocnemii, and the sciatic artery on one side ligated. The frog remained in the water an hour, and was then taken out, killed, the gastrocnemii removed and put into Ringer's solution. The loss in weight which they suffered indicated the degree of hypotonicity attained, i.e., the amount of water held by the muscle cells.

TABLE V

HOURS IN RINGER'S SOLUTION	ARTERY LIGATED WEIGHT OF MUSCLE IN MGM.	PER CENT LOSS	CONTROL CIRCULATION INTACT WEIGHT OF MUSCLE IN MGM.	PER CENT LOSS
0	2075		2005	
1	1910	8.0	1920	4.2
12	1815	12.5	1870	6.7

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Hence where the arteries are eliminated the water retained by the muscle is much greater in amount, showing the reduced efficiency of the veins.

From the experiments which we have described, it seems clear that, in the normal frog the water which is being continually absorbed through the skin is removed by the lymphatics and veins. When a limb is completely ligated, liquid rapidly accumulates under the ligature. This liquid is composed of transudate and of water absorbed through the skin by osmosis. Removal of the superfluous liquid is prevented because both veins and lymphatics are closed. The lymph therefore tends to become more and more dilute and the muscle correspondingly swollen with the continued absorption of water. In short, the phenomena of edema develop below a complete ligature because lymph and tissue take up water osmotically, and the avenues for its removal are blocked. In the case of edema produced by a lymphatic ligature, the lymph formed in the normal fashion is retained while the absorbed water passes into the capillaries and is removed.

Since experimental edemas in frogs have been used as a basis for various theories of edema, it has seemed worth while to attempt an analysis of a matter so fundamental. We have sought to determine in how far known factors would serve us in such an analysis.

Should these factors fail to account for all of the observed phenomena then it would be in order to form other working hypotheses. It has been shown that the simple factors, water absorption by osmosis, transudation of plasma and interference with the draining power of the lymphatics and veins, are sufficient to account for the phenomena of experimental edemas in frogs, and therefore further hypotheses are unnecessary.

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STUDIES ON LIGHT PRODUCTION BY LUMINOUS BACTERIA

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In this research I have proceeded upon the assumption that light production is due to the oxidation of a phosphorescing substance (photogen) in presence of water and free oxygen and some oxidizing enzyme, and have endeavored to extract the photogen with various solvents. The extraction experiments have failed but have at the same time led to some interesting results on the stability and solubility of the substance or substances concerned in light production. We must always bear in mind the fact that in dealing with a biological process such as photogenesis, requiring the presence together of at least two substances, photogen (A) and enzyme (B), in all probability, the solvent may extract only one of the two substances, let us say A, and we should not expect the extract to phosphoresce until substance B is added. If the solubilities of A and B are both unknown the interpretation of results is rendered difficult and sometimes uncertain.

Luminous animals may be divided into two classes—those in which the luminous material is burnt within the living cell (Firefly, Fungi) and those in which it is secreted by the cell and burnt outside (many worms, crustacea and myriapods). As first shown by Molish[1] the luminous substance must be burnt within the bacterial cell since a dense emulsion of the luminous bacteria may be separated from the medium by a Chamberland or Berkefeld filter and a clear dark filtrate with no trace of phosphorescence obtained. I have repeated Molish's experiment and can

[1] Molish: Leuchteude Pflanzen, 1904, p. 116.

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confirm him. An alundum filter crucible was used. Can this photogenic substance in the cell be freed of other cell material and obtained in a more or less pure state? We know that the bacteria can be dried, and when moistened again will phosphoresce, even though the majority are not living and will give rise to no new growth if inoculated in a suitable culture medium.[2]

These dried bacteria form an excellent material for extraction purposes. The method of growth, collection and desiccation is as follows. The organisms are best grown in bulk in a thin layer of peptone (1 per cent)-glycerine (1 per cent)-sea water nutrient fluid covering the bottom of a white enamelled pie plate and covered by another pie plate, the whole readily sterilized and serving as a large Petrie dish. The medium must be faintly alkaline to phenolphthalein. The bacteria are easily collected by centrifuging. The dense mass of centrifuged bacteria is then spread in a thin layer on filter paper or on glass wool, placed in a desiccator and dried over $CaCl_2$ in a vacuum. Spreading on glass wool has the advantage that the glass wool may be ground up in a mortar and a powder obtained which phosphoresces when moistened, but the powder does not give so brilliant a light as does the filter paper containing dried bacteria. It is well to wash the glass wool with several changes of water to remove alkali.

Strips of bacteria impregnated filter paper can be extracted with boiling ether or cold absolute alcohol for twelve hours without losing their power to phosphoresce when the solvent has been removed and they are again moistened. Colonies of luminous bacteria sometimes appear when the filter paper is placed on a nutrient medium even after such rigorous treatment with ether and alcohol and other fat solvents. Büchner and Gaunt[3] obtained similar results with the acetic acid forming

[2] I have on two occasions obtained no luminous growths from bacteria which had been dried on filter paper and afterwards moistened (light was produced) with sterile sea water and placed on nutrient agar culture medium. In the majority of cases, however, colonies of brilliantly luminous bacteria result. These colonies are relatively few in number indicating that most of the bacteria are killed by drying.

[3] Buchner and Gaunt: Liebig's Annalen, 1906, 349, p. 140.

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bacteria of beer (Mycoderma aceti). The dried acetic bacteria are not all killed by extraction with acetone; moist bacteria are invariably killed.[4]

These experiments show (1) that the photogenic material is not a fat or a fat like body soluble in fat solvents and (2) that phosphorescence does not depend on the living cell, since many of the dried bacteria which can still phosphoresce when moistened will give rise to no new colonies.

Phosphorescence does seem to depend on the integrity of the cell or a certain structure in the cell. All my efforts to break up the cell and obtain a phosphorescent solution failed. This result might have been anticipated by the work of Macfadyen[5] who found that luminous bacteria subjected to the action of liquid air did not phosphoresce at that low temperature but did phosphoresce as soon as warmed again; further—that if the cells were broken up by grinding at the temperature of liquid air, there was no phosphorescence or rewarming. Macfadyen worked, however, in the presence of oxygen and moisture and we might suppose that a slow oxidation—too slow to produce light—went on in the material broken up at low temperatures with consequent exhaustion of the photogenic material.

To prevent oxidation it is of course necessary to work either in absence of oxygen or in absence of water. In my experiments the moist bacteria have been broken up (cytolysed) in absence of oxygen by (1) oxygen-free distilled water and (2) toluol. All marine cells can be cytolysed by distilled water or fat solvents. The *dry* bacteria have been broken up by grinding with sand. The results on the moist material will be considered first.

In the first method a dense mass of bacteria are placed in a vessel from which the air is exhausted by an air pump.[6] The

[4] I find that extraction of dried luminous bacteria with 95, 80, 50 or 35 per cent alcohol (table 1), kills them all and no new growths appear. Also if the moist bacteria are treated with a large excess (50 volumes) of absolute alcohol or acetone for ten minutes and then rapidly dried no growth is possible. Bacteria so treated have also lost their power to phosphoresce when moistened.

[5] Macfadyen: Proc. Roy. Soc. 3, 1902, 71, p. 76.

[6] For the apparatus used see Harvey: Journal American Chemical Society, 1915, 37, p. 396.

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bacteria stop glowing but reglow if air is again admitted. Then oxygen-free distilled water is allowed to flow onto the bacterial mass and it is thoroughly shaken. No light appears (indicating that the water is oxygen-free) and five to ten minutes later if oxygen is added still no light is emitted. If there is a definite soluble photogenic substance in the bacterial cell it should have passed into solution in the water when the cell was cytolysed, and, provided no decomposition took place, it should have glowed when oxygen was readmitted. Even if we assume that the cell was not completely cytolysed, the photogen, if a stable substance, although one unable to pass the cell surface, should have glowed within the cell.

In the second method a dense emulsion of the bacteria in sea water is rendered non-luminous by removing the oxygen. Then a drop of toluol is added without admitting oxygen (air). The emulsion is shaken and no light appears. In a few minutes air is admitted and still no light appears. Similar experiments with ether, chloroform and carbon tetrachloride gave similar results. Thus if the cells are broken up the photogen disappears even though it has not been oxidized, for no oxygen was present. The toluol itself does not destroy the photogenic substance as evidenced by the treatment of dried bacteria with toluol. Luminous bacteria in *oxygen-containing* sea water to which a drop of toluol, ether, chloroform or carbon tetrachloride is added very quickly stop phosphorescing. I explain this as due to the fact that on cytolysis of the cell the oxidation processes run riot and the available store of photogen is rapidly used up. The same explanation may be applied to the loss of light in distilled water. There can be no question of a destructive effect of the cytolytic agent in the case of distilled water. We may compare the conditions in bacteria to the conditions in a potato cell. When the cells of the potato are crushed or when their surface is destroyed by toluol or ether or chloroform, dark melanin oxidation products are rapidly formed, but if the potato is cut and the cut cells well washed to free them of their cell contents, no blackening occurs, although the lower intact cells at the cut surface are exposed to atmospheric oxygen and only separated from it by their plasma

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membranes. A destruction of these membranes would immediately cause oxidations within to proceed rapidly.

The conclusion drawn from the above experiments has been confirmed by allowing *oxygen-free* sea water to come in contact with dried bacteria in a hydrogen atmosphere. If, after fifteen minutes, oxygen is admitted, no glow is observed although dried bacteria glow for a short time if moistened with oxygenated sea water.

All the above experiments, then, point to the conclusion that if the cell is broken up while moist or if the dead cells stand in contact with water for any length of time, *even though no oxygen be present*, nevertheless the photogenic substance undergoes decomposition, a conclusion corroborated by my work and that of McDermott on the fire-fly.[7] Extraction of the dried fire-fly luminous organs with oxygen-free solvents will give no phosphorescent solutions on admitting oxygen, because of this instability of the photogen.

In the normal living bacterial cell (or fire-fly cell) I assume the photogen to decompose through oxidation with light production. If the living bacteria are kept in sea water from which all oxygen has been removed and they stop glowing, they will still glow strongly if oxygen is readmitted, even after a period of twenty-four hours. It is therefore obvious that the breaking up of the photogen in absence of oxygen does not occur in the intact bacteria but only in those whose normal "structure" has been destroyed by cytolysis. I am inclined to believe that the surface layer of the cell is the "structure" involved.

The question may now be raised as to whether bacteria whose structure has been completely destroyed by grinding in the dry state will phosphoresce on moistening. Oxidation cannot take place so long as water is absent. Experiment shows that they will not phosphoresce as the following procedure indicates. Luminous bacteria dried on glass wool are powdered in a porcelain mortar and divided into two equal parts, A and B. A was then ground in the porcelain mortar for twenty minutes with

[7] Harvey: Jour. Amer. Chem. Soc. 1915, 37, p. 396 and Science N. S. 1914, 40, p. 33, also McDermott: ibid, 1915, 37, p. 402.

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pure quartz sand. B was thoroughly mixed in another mortar with an equal volume of sand, previously ground for twenty minutes, and exposed to the air during the time of grinding A. In this way the effect of quartz powder or the possibility of absorbing moisture from the air would be the same for the unground bacteria, B, or the ground bacteria, A. On moistening B with sterile sea water, a good phosphorescence appeared while the ground material gave no light with sea water. I have repeated the experiment with the same result and feel that there are no possible sources of error. Microscopic examination shows the sand to be ground to the size of the bacteria or smaller and it is well known that even the smallest cells may be broken up by grinding with sand. I find that the dried luminous organs of the fire-fly likewise lose their power to phosphoresce if thoroughly ground with sand. This result differs from that of McDermott[8] who finds that fire-fly tissue can be frozen and ground in liquid air without losing its power to phosphoresce. Both A and B were inoculated on agar nutrient medium. The ground bacteria, A, gave rise to no luminous colonies while the unground bacteria, B, did develop several luminous colonies, further proof that the ground bacteria were wholly broken up and destroyed.

In view of the statement often put forward that the photogen is a fat or a lipoid, many attempts were made to obtain something which would phosphoresce by extracting with fat solvents. All attempts have failed. The following table (table 1) gives the results. The strips of filter paper containing the dried bacteria were placed in the solvents in sterile tubes for a definite time at a definite temperature, the solvent completely removed and the filter paper tested for light production by adding sterile sea water. A + indicates light; a − indicates no light. Controls, untreated with any solvent, always gave a good light. In the last column are similar results obtained with the dried powdered luminous organs of the fire-fly.

The great majority of fat solvents extract nothing which is essential to light production. Chloroform might have extracted

[8] McDermott: Smithsonian Report, 1911, p. 345.

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something as the material glows only faintly after chloroform treatment. I have, however, evaporated the chloroform extract to dryness in vacuo and added water as well as a water extract of luminous bacteria (in itself non-luminous; possibly

TABLE I

SOLVENT	TEMPERATURE	TIME OF EXTRACTION	DRIED BACTERIA	DRIED FIRE-FLY
	degrees	*hours*		
Ether (cold)	20	24	+	+
Ether (hot)	35	24	+	+
Chloroform (cold)	20	12	+	+
Chloroform (hot)	61	12	+ faint	+
Ethyl alcohol (cold)	20	12	+	+
Ethyl alcohol (hot)	78.4	12	−	−
Alcohol and Ether (equal parts boiling)	46	12	− to faint light	+
Acetone (cold)	20	12	− to faint light	+
Acetone (hot)	56.3	12	−	+
Carbon tetrachloride	20	24	+	+
Carbone bisulphide	20	24	+ fair light	−
Toluol	20	12	+	+
Toluol (hot)	100	12	−	−
Benzol	20	12	+ fair	not tried
Benzine (Petroleum ether)	20	24	+ fair	+
Amyl alcohol*	20	24	+	+ faint
Ethyl butyrate*	20	24	−	−
95 per cent Alcohol	20	24	−	+ very faint
80 per cent Alcohol	20	24	−	−
70 per cent Alcohol	20	24	−	−
50 per cent Alcohol	20	24	−	−
35 per cent Alcohol	20	24	−	−
Material kept dry at	78.4	12	+ fair	+
Material kept dry at	100	12	−	−

*Washed with ether to remove solvents.

containing an oxidizing enzyme) to the residue without obtaining light production. The same result was obtained with the residue of the boiling alcohol extract, so that we must conclude that the chloroform and boiling alcohol extract nothing but rather destroy the photogenic material. The temperature of

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boiling alcohol, 78.4°, is not destructive to the photogen. These results are very similar to my previous results[9] on fire-fly material as may be seen by inspecting the last column. The photogen of the fire-fly is not weakened by chloroform or acetone or a boiling mixture of equal parts alcohol and ether, but does suffer from carbon disulphide. Otherwise the results are the same.

The strips of filter paper moistened with sterile sea water were then transferred under sterile conditions to nutrient agar to see if colonies of luminous bacteria would result. It was found that not in every case but in at least one experiment luminous colonies were obtained after extraction of the material with ether, alcohol, toluol, acetone and benzol.

I have already mentioned the fact that *dried* bacteria will glow if moistened after extraction with cold (20°) absolute alcohol and also the fact that if fifty volumes of absolute alcohol is added to a mass of moist centrifuged bacteria and they are then shaken for ten minutes, the alcohol removed and bacteria quickly dried, no phosphorescence is obtained on moistening this dry powder. Neither will dried bacteria phosphoresce if extracted with 95, 80, 70, 50 or 37 per cent alcohol. What is the explanation of this? The alcohol does not dissolve out a luminous substance. It is also well known that alcohol does not destroy the ordinary oxidases. They are precipitated by alcohol and will redissolve in water. It is however very possible that the oxidizing enzymes in luminous bacteria are similar to the "oxydones" investigated by Batelli and Stern[10] which are destroyed by alcohol and acetone. Or perhaps we may explain the above result as merely an example of the effect of alcohol on dry albumins as opposed to albumin solutions. Powdered egg albumin can be extracted with absolute alcohol (or acetone) for six hours and is still readily soluble in water. But if a concentrated aqueous solution of albumen is precipitated by a large excess of alcohol (or acetone) even though the alcohol (or acetone) be removed within fifteen minutes the precipitate of albumen is found to be practically insoluble in water. It would be futile

[9] Harvey: Jour. Am. Chem. Soc., 1915, 37, p. 400.
[10] Batelli and Stern: Bioc. Zeit., 1914, 67, p. 443.

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to discuss the matter without further experimental results but I think we may say that in photogenesis there is involved a substance which in the moist state is irreversibly precipitated (or changed) by alcohol and that it is probably protein in nature.

SUMMARY AND CONCLUSIONS

1. Luminous bacteria which have been rapidly dried over calcium chloride in a vacuum will phosphoresce if moistened with *oxygen-containing* water but not if moistened with *oxygen-free* water. Drying does not kill *all* bacteria but does kill most of them. Hence phosphorescence does not depend on the living cell.

2. Dried bacteria, if finely ground with sand, will no longer phosphoresce when moistened. None of the ground bacteria can grow. Phosphorescence does depend upon the integrity of some structure in the cell.

3. Dried bacteria extracted with ether or toluol will still phosphoresce if moistened and may develop colonies on a suitable culture medium. Consequently neither ether nor toluol destroy the photogen.

Bacteria in *oxygenated* sea water to which ether or toluol is added stop phosphorescing, presumably because the photogenic substance is rapidly oxidized and used up when the bacterial cell is cytolysed.

Bacteria in *oxygen-free* sea water do not glow, but will glow if oxygen is admitted, even after a period of twenty-four hours. Bacteria in *oxygen-free* sea water to which toluol or ether is added will not glow if oxygen is readmitted after fifteen minutes. Hence the phosphorescent substance undergoes decomposition in the absence of oxygen, a decomposition not due to the toluol (compare the first statement in section 3), but probably due to enzyme action since toluol does not affect the action of enzymes.

4. Moist luminous bacteria to which *oxygenated* distilled water is added cease glowing, presumably because the photogen is rapidly oxidized and used up when the bacterial cell is cytolysed.

Moist bacteria to which *oxygen-free* distilled water is added will not glow even momentarily if oxygen be readmitted after

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fifteen minutes, a result again pointing to instability of the photogen when the cell structure is affected by cytolysis.

5. Dried bacteria placed in *oxygenated* sea water phosphoresce momentarily, but if dried bacteria stand in contact with *oxygen-free* sea water for fifteen minutes, no phosphorescence occurs when the oxygen is admitted. Again (as in sections 3 and 4) the photogen has decomposed. It is therefore impossible to extract a phosphorescent substance from bacteria with *oxygen-free* aqueous solvents.

6. Fat solvents extract nothing which will phosphoresce from the dried bacteria. Some of the bacteria survive and will grow after such extraction. Boiling alcohol, cold acetone and ethyl butyrate destroy the power to phosphoresce.

7. Dried bacteria do not lose their power to phosphoresce after twenty-four hours extraction with cold absolute alcohol, but moist bacteria (centrifuged) treated with fifty volumes of absolute alcohol, and then dried rapidly will not again phosphoresce if moistened. The enzymes concerned in light production are consequently of a wholly different nature from the ordinary oxidases which are not destroyed by alcohol or acetone.

I am at present engaged upon a study of the oxidizing enzymes of luminous bacteria and expect to publish the results in a short time.

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CARDIAC INHIBITION DURING THE VOMITING EVOKED BY STIMULATION OF THE GASTRIC VAGUS

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In a paper published a few years ago[1] I showed that a fall of blood pressure occurs during the vomiting induced by faradization of the gastric branches of the vagi. The experiments reported were performed on cats anaesthetized with chloralose (Merck), an anaesthetic which, as was shown,[2] greatly facilitates the elicitation of the vomiting. Since the vomiting evoked under these conditions is peculiarly prolonged there is an especially good opportunity to study the associated blood pressure changes. The lowering of the blood pressure in the experiments mentioned was not accompanied by any slowing of the heart (see figs. 10 and 11 in paper referred to). The effect must, therefore, have been produced either by the mechanical influence on the circulation of the muscular movements of vomiting or as a result of vaso-dilatation. On general grounds the mechanical mode of causation appears the more probable.

It is somewhat surprising that no inhibition of the heart should accompany vomiting when we consider the powerful character of the discharges occurring from the vomiting center. The explanation is probably to be found in the fact that the vagus of the cat contains relatively few cardio-inhibitory fibers as compared with other animals. I, accordingly, performed some similar experiments on the dog in order to see whether any cardiac inhibition would result.

[1] Miller: Pflüger's Archiv, 1911, cxliii, 21.
[2] Miller: Pflüger's Archiv, 1911, cxliii, 1.

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The technique of these experiments was identical with that previously employed. Chloralose was used as the anaesthetic, about 15 cc. of a 0.2 per cent solution being injected intravenously per kilo of body weight. The animal breathed through a short tracheal cannula into a large bottle, the other opening of which was connected to a Marey's tambour. This arrangement served to record not only the respirations but also the movements of vomiting. In this article, as in the previous one, the term vomiting is applied to the muscular movements, the essential element of the act, and not to the discharge of material from the stomach. The blood pressure in the carotid artery was recorded with a Hürthle manometer.

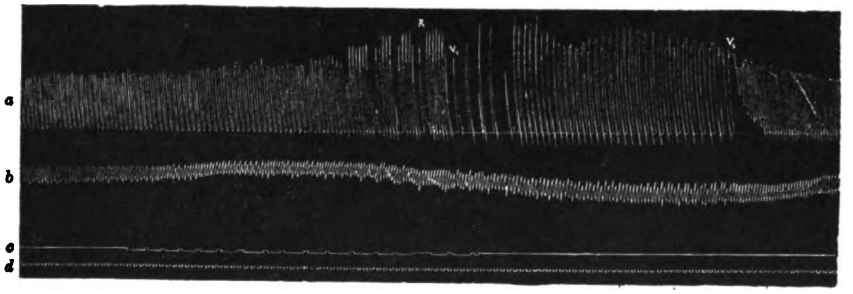


Fig. 1. Record of vomiting and blood pressure on stimulating dorsal gastric vagus. Sec. dist. 12 cm. *a*, respirations and vomiting; *b*, blood pressure; *c*, signal; *d*, time in seconds; *R*, retching; V_1 - - - V_2, vomiting.

A record of the vomiting movements and associated blood pressure changes is shown in figure 1.

The dorsal branch of the gastric vagus was stimulated repeatedly with brief intermissions until the reflex was excited. At *R* the respirations were interrupted by a retching movement. Between V_1 and V_2 vomiting occurred. There was some slight cardiac slowing during the retching but marked inhibition took place in the course of the actual vomiting. Other records were repeatedly obtained which showed stronger cardiac inhibition. The essential results are, however, sufficiently clearly exhibited in the record reproduced (fig. 1).

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As has just been stated the inhibitory effect is greatest during the vomiting movements. Since the stimulation of the vagus was discontinued as soon as vomiting began it is clear that the cardiac inhibition is produced by the vomiting *per se* and not by the nerve stimulation. Hence it appears that in the dog the vomiting center when active is able to influence the cardio-inhibitory center in a powerful manner. A similar conclusion was recently arrived at by Brooks and Luckhardt,[3] who observed cardiac inhibition during the vomiting excited by various emetic substances.

We may conclude from the results reported above that the fall in blood pressure during vomiting in the dog, while, no doubt, in part produced by the factors effective in the cat, is materially increased by cardiac inhibition.

[3] Brooks and Luckhardt: American Journal of Physiology, 1915, xxxvi, 104.

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THE MACROPHAGES OF MAMMALS[1]

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Although in the last few years a vast amount of careful scrutiny has been accorded the blood cells of mammals and in addition the cells which normally inhabit the connective tissues, few or no new general view-points or new bases for classification for these cells have arisen. It is then of especial moment that recent work does afford such a general view-point concerning one of the cell types abundantly present throughout the body, a type probably of supreme importance both in the defense of the body and in its normal physiological mechanism. To these cells I propose to give the term "macrophage," appreciating well that the most varied structures have been included under this term and that a general lack of precision has attended its use ever since Metschnikoff[2] proposed the word years ago. Concerned chiefly in another thesis Metschnikoff even in his last general presentation[3] has not sought cytological criteria for the separation of these cells from the mononuclear blood elements.[4]

[1] Sometime ago, inquiries into the nature of endothelium led the writer to experiment extensively with acid azo dyes, a class of bodies which had not previously met with much biological use. These dyes are stored in a practically specific manner by certain mononuclear cells which constitute a great tissue or organ. Recent communications, especially by Aschoff and his pupils, are beginning to confirm this view and a summary of the body of well established facts which may now justly be regarded from a general point of view, would seem justifiable. The present communication was read in an abbreviated form before the Society for Experimental Biology and Medicine, New York, October 21, 1914.

[2] Metschnikoff, Elias: Leçons sur la Pathologie comp. de l' inflammation. Paris, 1892.

[3] Metschnikoff, Elias: Die Lehre von den Phagocyten, Kolle u. Wasserman, Handb. d. path. Mikrorganismen, Zweite Auf. II, p. 655, 1913.

[4] Metschnikoff, Elias: Die Lehre von den Phagocyten, Kolle u. Wasserman, Handb. d. path. Mikrorganismen, Zweite Auf. II, p. 674, 1913. (See for instance his acceptance of round cell infiltration as a collection of young phagocytes.)

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It is a singular commentary on the separation of interests that there has been no adequate anatomical inquiry of just what and where were the great phagocytes which Metschnikoff insisted upon as a cell class. Yet for years the slow accumulation of data on the behavior of the body towards foreign substances has brought these cells to our notice. These were met with above all in the great serous cavities, the pleura, the pericardium, and the peritoneum, especially after the introduction of foreign bodies. They formed the foreign body giant cells in the skin and they were no less evident in the lymph glands of anthracosis. Then the direct intravenous introduction of more minute foreign bodies in the form of suspensions called forth these cells again, for singularly enough the blood current itself was lined by these cells in the liver, bone-marrow and spleen.

In all these cases we were confronted by great mononuclear cells whose dimensions and structure departed far enough from the blood mononuclears to compel a distinction, and yet that distinction was not made. All possible transitions (Uebergangsformen) were secured to align them with the customary blood cells of their kind (lymphocytes and large mononuclears).

It might long ago have occurred to the investigator to pursue farther ideas suggested by the reaction of these cells to foreign bodies of whatever size. The immediate problem here would be but to find a substance not acted upon chemically by the body and yet so finely divided as to actually have diffusion powers so as to reach the abundant macrophages which are outside the blood vessels. Fortunately substances in this state exist, for the colloids answer our criteria. Some hydrosols possess particles large enough to have measurable physical dimensions and yet small enough to possess some of the properties of solutions, especially to diffuse. Fortunately a large number of dyestuffs[5] which are without toxic or indeed any appreciable

[5] I have elsewhere discussed at some length the chemistry of the dyes to which reference is here made (see Evans and Schulemann: The action of vital stains belonging to the benzidine group, Science N. S. xxxiv, No. 1004, pp. 443–454, March 27, 1914). It is sufficient to instance trypan blue as a dye typical of this class.

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action on the body form brilliantly colored colloidal solutions. When introduced into the living animal by practically any route, subcutaneous, intraperitoneal and intravenous, these dyes quickly diffuse so as to give an intense general stain to the tissue. The dye at first exists merely in the body fluids giving also a uniform stain to the elastic and other connective tissue fibres and to various membranes. The elastica of arteries, the dura and the zona pellucida of ova, are excellent examples here. Soon however the dye is found housed in specific cells, always chief among which are the macrophages. In the cytoplasm of these cells, the dye is concentrated in granules of differing intensity and dimensions, scattered irregularly. Frequently the dye exists in the watery fluid of large vacuoles in which concrements of dye dance in the liveliest Brownian motion. Under no circumstances does the living nucleus store the dye. The granules and vacuoles in the protoplasm, however, are not preformed structures, stained by combining with the dye, but are themselves produced as the result of the entry of the dye into the cell which disposes of it in this way. It is doubtful whether any other method would so decisively have persuaded us to class together the cells which we now know as macrophages, for the class, singularly enough, not only includes both tissue-forming sessile and free cells but also crosses some rather fundamental cell categories embracing as it does endothelial and connective tissue cells. However, the identity of nature of these diversely appearing elements is proven not alone by their specific reaction to the vital stain but also by the actual conversion of the sessile cells into the free ones under spontaneous as well as experimental conditions.

Morphological characteristics of macrophages. It may be objected now that the concept which has just been formulated of the macrophages, is not a substantial morphological one, but that we have taken a rather general physiological trait of cells and used it to unite together very diverse elements into a common class. A terse description of these cells is called for, consequently, for the macrophages have a characteristic morphology as well as function and are produced by elements essen-

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tially similar, not diverse, in type. Yet should future inquiries ever throw together genuinely diverse cell types, one could still inquire with reason why this circumstance should give distress, for some agreements in the behavior of cells and tissues are biologically more important than their differences. Most earnestly might we ask whether such tests do not afford a deeper basis than our old ones for the classification of cells.

The macrophages of mammals are mononuclear cells, usually relatively large yet varying in size from about 10 microns to several times this size and being well rounded or very elongated elements in accordance with their surroundings and whether they be free or sessile cells. The nucleus, excentrically situated, possesses a prominent membrane against which most of the scanty chromatin lies and which is seldom regular in contour, being less convex or in fact concave toward the main cytoplasmic mass. It is at this side of the nucleus, in a relatively clear "hof" of cytoplasm, that the micro-centra lie. When certain basic stains (brilliant-cresyl-blue, methylene blue, neutral red) are applied supra vitally to these cells, their somewhat smaller nuclei stain with greater rapidity than do the nuclei of fibroblastic cells and are much more deeply-tinged substantial structures than the delicate oval nuclei of the latter. The cytoplasm, almost always abundant, yet variable in amount, shows itself in reaction to well known blood-stains (Giemsa, Unna) as weakly basophilic: towards gentian violet it may behave metachromatically. Schultze's oxydase reaction is negative. A delicate reticular structure (Pappenheim, Kiyono) of the cytoplasm, evident after fixation, is to be referred to coagulative changes, as the study of the fresh unfixed cells shows. When examined on the warm stage and in the body fluids, the cytoplasm of these cells never reminds one of reticular structures but is often honeycombed with minute vacuoles and granules interspersed with larger structures of the same type. The diversity of these structures may be remarkable and their occurrence is highly characteristic of the macrophages. Still another point in the structure of these cells is almost entirely lost in fixed and stained preparations but is always evident in the warm and living cells.

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I refer to the abundant, various sized, and often delicate pseudopodia which may cover their surface.[6] These are by no means similar to the delicate winglike or pointed processes of the fibroblasts. Their activity shows them to be true pseudopodia and it is somewhat remarkable to see many of these small structures protruding from the thin cytoplasmic shell of macrophages which are enormously distended by vacuoles.

Distribution of the macrophages. The macrophages which are endothelial in nature line the following: capillaries of the hepatic lobules, capillaries and venules of the spleen, capillaries and venules of the bone marrow, capillaries and venules of the haemal glands, lymphatic sinuses of the lymphatic glands.

Other macrophages which are more or less fixed cells include: First, cells which in appearance and probably origin are true endothelial cells, not concerned however in vascular channels. Chief among these are the well known reticulum cells of lymph glands and similar cells in the splenic pulp and bone marrow. Second, cells not directly related to endothelium, chief among which are the great mass of cells in the connective tissues designated variously as clasmatocytes or resting wandering cells. Rather rounded free strains of these cells occur in the omentum where they form the colonies known as taches laiteuse.

The free macrophages comprise the characteristic mononuclear cells of the serous cavities, similar mononuclear cells often abundant and always present in the lymphatic sinuses of lymph glands and similar cells sometimes present in the splenic and hepatic capillaries and under rarer conditions in the peripheral blood stream.

A brief discussion of the macrophages under the above heading will not be out of place. The phagocytic nature of the endothelium of the liver has been well known for a long time, Kupffer first calling attention to the regular overgrowth of certain of these cells into larger elements (Kupffer cells) and establishing their true endothelial nature. Where the liberation of

[6] Kiyono, for instance, could hardly have availed himself of the method of direct study of the living cells. Vide his figures and statement—"der periphere Rand des Protoplasmas ist glatt."

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blood or bile pigment is extensive (e.g., malaria) the engorgement of these cells with the pigment is well known and they are equally active in the engulfment of red cells[7] and bacteria.

A similar phagocytic activity on the part of the splenic endothelium is also by no means a new fact. It is a fact that in cases in which the spleen undergoes a marked pigmentation, the pigment is not bourne only by characteristic large cells of the pulp (free macrophages), but also by some of the endothelial cells lining the venous sinuses. I shall recur to cases of the abnormal production of these splenic macrophages later.

The bone marrow, the capacity of which to store pigment normally has been gradually recognized and recently made the basis of Brass' [6b]paper, shows a similar endothelial activity. But though one may, after search, satisfy oneself that endothelium is concerned here, there are likewise in the marrow other macrophages whose connection with the endothelium is not clear, a fact partly due to our ignorance of the exact form of the vascular tree at this point, but also perhaps to the possibility that, just as in the lymphatic glands, endothelial cells, independent of vascular channels, form a regular component of the tissue. These cells, the so-called *reticulum cells*, have long been recognized as having characteristics which allied them to endothelium. It is of interest that the vital stain confirms the notion that these cells belong to our general category.

The lymphatic sinuses of lymph glands and the venous sinuses of haemal glands are lined with endothelial cells whose activity as macrophages is pronounced. The intravenous injection of azo dyes or colloidal metals causes these cells to be densely loaded. This participation on the part of the endothelium, however, is sharply limited to those definite tracts of it well within the haemal and lymphatic glands. The entering or draining trunks, in the case of the lymph gland, do not show any pecul-

[6b] Brass: Ueber physiologische Pigmentablagerung in den Kapillarendothelien des Knochenmarks, Arch. f. mik. Anat. 1913, Bd. 82.

[7] Kyes has shown that in the pigeon the Kupffer cells phagocytise erythrocytes which appear entirely normal. Similar "hemophages" exist as endothelium in the spleen. Kyes, Preston: Morphological evidence of intracellular destruction of red blood corpuscles, Proc. Am. Assn. of Anatomists, Anat. Record vol. 9, no. 1, p. 97.

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iarity (enlargement, phagocytosis, etc.) on the part of their lining cells; whereas, in the case of the haemolymph glands, nothing is more striking than the abrupt assumption of brilliant dye granules by the endothelium of a venule just as it enters and resolves itself within the gland.

The other macrophages which must be counted as regular tissue components are those large cells of the connective tissues which have been variously designated as rhagiocrine cells (Renaut), clasmatocytes (Ranvier), adventitia cells (Marchand), resting wandering cells (Maximow), and pyrrhol cells (Goldman). The characteristic cytology of these cells is too well known to need description here. They have always been known as prone to contain a variety of granules and irregular inclusions and to display vacuoles,[8] an extreme content of which leads to a foam structure. The activity of these cells in inflammatory conditions as great phagocytes is equally well known. Although these cells may be provided with long processes, under only slightly changed conditions this expansive tendency is withdrawn. The cells speedily "round up" so as to possess considerably more delicate pseudopodia and to be identical with the free macrophages of the serous cavities. The omentum, whose activity towards foreign bodies in the peritoneum is so pronounced, owes this largely to its great content of these cells, colonies of which form the cell clumps known as taches laiteuse.

Under normal conditions by far the greatest number of free macrophages is represented by the army of peculiar large mononuclear cells which inhabit the serous cavities. Schott has recently subjected these cells to a critical examination, and it is scarcely necessary to recount the conflict of views on their nature and origin. All available hypotheses for their origin have

[8] Perhaps attention may be called here to the tendency of French histologists (Renault and his pupils) to see a special internal secretion of the connective tissue elaborated by these vacuoles. Enough is known to indicate that vacuoles may be the expression of intracellular digestive processes or true excretory as well as secretory phenomena. Renant, J.: Les cellules connectives rhagiocrines, Arch. d' Anat. Micr., t. ix, Fasc. iii-iv, 1907. Dubrenil, G., Le chondriome et le dispostif de l'activite secretoire, Arch. d' Anat. Micr., t. xv, Fasc. i, 1913.

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been stoutly maintained. They have been claimed for desquamated mesothelial cells, for endothelial cells, connective tissue elements or emigrated blood mononuclears. Weidenreich in his last general monograph derives them both from mesothelium and connective tissue and is willing to state that "die Macrophagen der Trans-und Exudate der serosen Höhlen identisch sind mit den grossen ungranulierten Leucocyten des Blutes und der Lymphe."[9] Besides the more typical great transudate cells of these cavities there exist always much smaller mononuclear elements difficult indeed to distinguish from the lymphocytes of the blood. These young macrophages, for a continuous series of cells shows them to be such, may store granules of the vital stains, certainly a negation of lymphocytic affinities. The whole series of these cells which live free in the great cavities, does not only seem independent of the blood cells but is also in no way connected with the mesothelial cells lining the cavities. The oft repeated contention that desquamated mesothelium is concerned here is decisively answered by the vital stain. The mesothelial cells in animals treated with vital azo dyes always show a sparse fine granulation, the tiny dye deposits being scattered in a ring peripheral to the nucleus of the cell. Larger granules and vacuoles do not occur, a precise distinction from the macrophage series.[10] Transitions do not occur. The mesothelium does not loosen itself and gradually change its character. The only fixed macrophages near enough at hand to produce their free brethren of the peritoneum are those so abundantly resident in the omentum, mesentery and subperitoneal connective tissues.

Every pathologist is aware that the sinuses of the lymph glands and the venous and capillary spaces of the spleen often contain peculiar large free phagocytic cells. Occasionally the liver capillaries are found with similar cells. These are the free intravascular macrophages which, however abundant in the vessels of

[9] Weidenreich, F.: Die Leucocyten und verwandte Zellformen Wiesbaden, 1911, p. 136.

[10] Minot's distinction of the mesothelium from the endothelium is thus supported in the clearest way by the vital stain.

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specific organs, never circulate in great quantities in the general blood current.

Origin of intravascular free macrophages. The problem of the origin of free intravascular macrophage cells is relatively simple for they can be seen in the act of emergence from their parent tissue. This is the case with the free macrophages which so often throng the sinuses of the lymphatic glands and with those which arise here and there within the venous sinuses of the spleen and which somewhat more rarely liberate themselves in the capillaries of the liver. In all three localities the direct origin of rounded cells from the neighboring endothelium can be demonstrated by stages of half separation. The experimental production of these cells can be accomplished by any agents which induce endothelial response. The rapid production of endothelial macrophages in the liver, for instance, follows liberation of tubercle bacilli in the portal blood current. But if either colloidal dyes or even suspensoids be let into the general blood stream over a long period of time, the stimulus is sufficient to give us a condition finally in which the vessels in all of the organs bearing specific endothelia (liver, lymph glands, haemal glands, marrow, spleen) are thronged with endothelial macrophages. Under such circumstances, of course, it inevitable that many of these are kept in the general circulation and nothing is more striking than to recover the great dye-bearing cells in the ear vein blood.

Importance of macrophages in pathological processes. The rôle of endothelial macrophages in pathological processes will probably become increasingly evident. Their response in haematogenous infections is particularly pronounced. The epithelioid and giant cell of the miliary tubercle, whose origin was so long disputed, are pure colonies of these cells.[11] It is, in fact, a dis-

[11] Evans, Winternitz, Bowman: Die vitale Faerbung des Tuberkel, Centralbl. f. Bakt. Abt. I. Orig., 1912, lxv, 403; and An Experimental Study of the Histogenesis of the Miliary Tubercle, Journ. Exper. Med. xix, 3, 1914. It is interesting that Goldman so impressed by the coelomic macrophages as to neglect the endothelial members of this class ascribed the formation of hepatic tubercles to a wandering thither of the great free peritoneal cells.

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tinct trait of the endothelial macrophages to form giant cells and syncytial plaques.[12]

That many pathological conditions are regularly attended by the over production of great free macrophages, hardly demands serious exposition.[13] This is not only the case in infections (see for example the behavior of the lymph-glands in typhoid fever,[14] anterior polio-myelitis, etc.), but also in other conditions of unknown origin. (Some of the splenomegalies[15] belong here.)

Macrophages do not occur in the peripheral blood stream

[12] Very evident in thromboses for instance. The giant cells seen in coccidiosis of the rabbit's liver as well as those occurring in practically normal animals, full of blood or bile pigments, are of this class.

[13] A recent exposition of the role of macrophages in pathological processes may be found in Professor Mallory's book (Mallory, F. B.: The Principles of Pathologic Histology, Saunders, 1914). To Mallory undoubtedly belongs the credit of having recognized the direct participation of endothelium in lesions of the lymph glands, liver and spleen particularly. The wider inferences which Mallory has drawn are unjustifiable. He has not hesitated to label macrophages wherever they may occur as "endothelial leucocytes," and is willing to take the somewhat remarkable, if logical, consequences of this conception by stating that "endothelial leucocytes number from 2 to 4 per cent of all the white corpuscles" and when present in cellular clumps in lesions have originated solely by emigration from the blood and lymph vessels or by the mitosis of such emigrated cells. The normal abundance of extravascular macrophages in the subcutaneous tissues of the entire body (the clasmatocytes) does not force us to account in any unusual way for their origin there, while the practical absence of macrophages from the normal circulating blood has already been mentioned. With no criteria other than those furnished by ordinary stains, sarcomatous giant cells and osteoclasts (p. 38 loc. cit.) might readily be designated "endothelial leucocytes" but until the specific reaction here shown as characteristic for macrophage tissue is given by these cells, caution must be exercised in a statement of their affinities. While the vital stain substantiates the conception that the endothelium in five organs (lymph glands, liver, spleen, bone marrow and hemal nodes) readily produces free intravascular macrophages, it does not support the contention that the extravascular macrophages have arisen in this way. We must be grateful for the attention which Mallory has drawn to the role of macrophages in many pathological processes, for his endothelial leucocytes are macrophages. The hypothesis of the purely endothelial origin of these cells is clearly untenable. The distribution of macrophages and their almost ubiquitous presence can be established by the vital azo dyes or similar methods mentioned in this report.

[14] Rindfleisch's "typhuszellen" (Marchand, loc. cit. p. 53). Mallory, F. B.: A histological study of typhoid fever. Jour. Exper. Med., 1898, iii, 6. Saltykow, S.: Ueber die sogenannten Typhuszellen, Zeitsh. f. Heilkunde, 1900, xxi, 10.

[15] See for instance, Gauther's Disease, large-cell splenomegaly, an enormous production of splenic macrophages. See Brill and Mandlebaum, Amer. Jour. Med. Sci., vol. 166, no. 6, p. 863, December 1913.

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except when produced in numbers so great that the condition may be called pathological.[16] It is true that by giving dye over a considerable time interval, the endothelial free macrophages[17] can be drawn in fair abundance into the circulating blood. Winternitz and I found that this phenomenon does not occur until after a definite time interval and dosage of the dye, an interval sufficient in each case for the proliferation from the mother endothelium to take place. No better proof could be adduced to show that normally these cells are not in actual circulation for were this the case, they would be evident, only a few minutes being necessary for the Kupffer cells to display an appreciable quantity of the dye. Nor do our experiments indicate that the leucocytes are converted into these cells, for steps in that conversion should immediately take place, the cells, being ideally accessible to the dye in which they swim.

Under pathological conditions there is no question that macrophages, even in considerable numbers, may appear in the peripheral blood stream, but they have been almost entirely overlooked. However, a casual search of haematological literature has thrown two or three instances of the clinical occurrence of circulating macrophages into our hands. The observers finding themselves in the presence of a cell foreign to the blood, exercised a not unreasonable caution in its identification.[18]

Finally it may be noted that the capacity of endothelium to produce free macrophages is not limited to the specific endothelia. Occurrences which place the endothelium of the most various vessels under unusual conditions, such, for instance, as the direct injury of the endothelium, cessation of the adjacent

[16] I cannot agree with Aschoff and Kiyone (Aschoff and Kiyone, Verhandl. d. Deutsch. Path. Gesellsch. 1913, 16 Tag. and Folia haematol. 1913, Bd. 15), who although speaking with some reserve, are nevertheless, anxious to establish the regular content of the circulating blood in these cells. So insignificant is this content that thousands of careful smears may be counted without macrophages making their appearance.

[17] Aschoff has preferred to call the blood macrophages "histiocytes," but since no evidence exists for belief that tissues other than the endothelium are concerned in their production, they are more precisely designated "endotheliocytes," if a term be sought to specify them as a distinct class. (Evans: Anat. Record, viii, 2, 1914.)

[18] Van Nuys, F.: An extraordinary blood, Boston Med. and Surg. Jour. clvi (1907), p. 390. Bartlett, W. B., Pub. Mass. Gen. Hos., vol. ii (1908), p. 390.

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blood current, in short in all cases of thrombosis and embolism, lead to the proliferation of endothelium. Batchelor and the writer have established this through experimental injuries on the larger vessels. Furthermore the tendency of the endothelium of vessels in the midst of inflammatory areas to "fatten" and become actively phagocytic is well known to pathologists. This potentiality of the endothelium is consequently very widespread. Probably no area of the body can be excluded in this respect. The behavior of the central nervous system would seem to justify this statement. In animals all of whose tissues bear an intense vital stain, this organ is singularly unaffected. Neuropathologists have shown us, however, that in lesions here, the glial cells awaken and transform themselves into macrophages with ease, and in analogy with this fact, the endothelium of neural vessels, though probably least inclined to do so among all the endothelial tissues, may occasionally undergo the same changes which vessels in the midst of inflammatory areas do elsewhere in the body.[19]

Appearance of macrophages in the embryo. Genetically there is no reason to suppose that the macrophages represent a very recent or highly specialized cell class. The behavior which is characteristic of them is a very general attribute of protoplasm and they may be said only to have developed it to a very high extent. We would expect them fairly early in the embryo and such is in fact the case.[20] Hoffbauer, long ago, located them unwittingly in the attention he called to peculiar large cells in the chorionic villi. Here, in human embryos, they occur remarkably early, perhaps as early as any considerable mesoderm is laid down in the membranes. But one may see them scattered sparsely well throughout the general mesenchyme of the embryonic body at stages of from 15 to 20 mm., when they are so prominent and characteristic as to have departed far from

[19] MacCurdy and the writer have shown that the Körchenzellen are typical macrophages, reacting to the colloidal acid dyes in a typical way. MacCurdy u. Evans: Experimentelle Laesionen des. Centralnervensystems, untersucht mit Hilfe der vitallen Faerbung, Ber. klin. Woch. 1912, Nr. 36.

[20] Kiyono remarks that the macrophages must be regarded as "eine in extrauterinen Leben des Kaninchen differenzierte Zellart!" Kiyono, K.: Die vitale Karminspeicherung, Fischer, 1914.

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other simpler embryonic cells.[21] Further study of them in vitally stained embryos is in progress.

Fundamental physiological characteristics of macrophages. Were the pronounced reaction of the macrophages to the vital azo dyes a phenomenon shown in the case of the dyes alone it would be difficult to explain. It might readily be urged that the dye stuffs are sufficiently similar to the body stuffs and complex enough to enable a subtle union of protoplasm and dye-molecule to take place. Ehrlich has in fact not hesitated to champion this notion, but the point is one which fortunately can be decisively settled. The slightest chemical change which would upset the relation of auxochrome to chromophore in these dyes, would shift the color of the dye appreciably or more probably destroy it altogether. Yet if it be imagined that these relations are maintained, it must still be possible surely to locate the chemical group, or better the configuration, which enables the reaction to take place. These ideas have, I believe, been sufficiently disproven and the vital staining with the azo dyes shown to stand in brilliant analogy with the fundamental functional peculiarity of these cells.

The very fact that a great number of chemically diverse dyes (carmine, isamine blue, trypan blue) lodge with equal uniformity in the bodies of the macrophages, would make one skeptical of the above notion. Schulemann and I have examined critically almost the entire number of possible dyes made from benzidine and similar bases united with the sulphonic acids of naphthylamines, naphtols and amido-naphtols and have shown that the production of a vital stain with these dyes can not be said to depend on any special component or chemoceptor of the dye molecule, but is solely a function of the physical state of the dye solution, dyes approaching a true solution in character, being brilliant vital stains, whereas, those which are most highly colloidal cannot gain a sufficiently general distribution to produce such an effect. It is easy to understand that in the former cases, the dye particles (amicrons, submicrons, or larger

[21] Essick has described the great activity of these cells in the peculiar cavities which occur in the embryonic corpus striatum. Essick, C. R., Publications of the Carnegie Institution of Washington, Contributions to Embryology No. 6.

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complexes as the case may be) are able to diffuse sufficiently to rapidly escape the blood vessels and present themselves to the immense mass of extra endothelial tissues of the body. Yet even those dyes approaching the true suspensoids in physical character are engulfed in exactly the same way by the macrophages providing they gain contact with these cells, as is, of course, the case to a limited extent in any method of application of the stain. But intravenously, the suspensoids are solely accessible to the endothelial macrophages, intraperitoneally, solely to the coelomic macrophages and subcutaneously, solely to skin macrophages of the immediate locality. Even the larger ultramicroscopic particles of these dyes, then, are received into the macrophages with avidity. The employment of true suspensions of coarser and coarser particles of any sort does not change this result. Suspensions of gold, silver, carbon, bacteria and cells share the same inclusion into the adjacent macrophages and it consequently appears conclusive that we are dealing in the whole series of these instances with a reaction to particulate matter, a reaction intimately connected with surface tension. The process known as phagocytosis has been adequately established as dependent on these forces yet it was felt that the limits of such a phenomenon hugged closely ordinary microscopic vision. The work with colloidal metals and with colloidal dyes, where with the ultra-filter and other means, we can approximate the actual size of the particles involved, shows us that the conception of phagocytosis, at base an adsorptive act, must be pushed into the ultramicroscopic realm. These considerations show that the macrophages are physically different from epithelial or other cells and their physiological importance is not decreased but rather increased enormously by the fact that not only bodies of diverse size but also of the most diverse chemical class can gain entrance to them in this way.

It is already clear that several bodies of biological importance are handled by the macrophages. Among these are the blood and bile pigments and the fats and fatlike bodies. I have already referred to the body of evidence which has now accumulated to show that a normal or physiological deposition of blood pigment takes place in the specific endothelia which we have men-

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tioned. The experimental introduction of blood pigment in quantity into the body leads to a similar deposition of it.

As to the storage of fat, cholesterin and other compounds of this series, suffice it to say that Anitschkow has demonstrated that it is in precisely these cells, both endothelial and extra-endothelial, that the storage takes place. Pathologists have long been familiar with the occurrence of large macrophages loaded with fat particles in the granulation tissue of suppurating wounds. Aschoff and Windauf have recently shown that instead of neutral fat, these granules consist of doubly refractive lipoids belonging to the cholesterin-ester group and Anitschkow has been able experimentally to call forth these large so-called pseudo-xanthoma cells by the introduction of staphylococcus cultures followed by foreign bodies into the skin. Similarly, precisely these cells, filled with anisotropic lipoids, constitute the xanthoma and xanthelasma cells, and it is especially significant that animals fed a long time with cholesterin suffer a very general conversion of their wandering cells into xanthoma cells.

It is now more than probable from direct observations which we are able to make with the ultramicroscope[22] that all members of the fat series exist in the body fluids in the form of true ultramicroscopic emulsions. These substances also, then, constitute colloidal systems, and undoubtedly the identity in the behavior of the macrophages towards such diverse matter can be referred back to the fact that in all these instances we are dealing with particulate matter.[23]

Definition of macrophages. All of these facts justify recognition that the great mass of mononuclear cells which we have described constitute a sharply defined cell group or class. *The macrophages may now be defined as those mononuclear cells, wherever they may be, lining vascular channels, resident in the connective tissues or entirely free, whose protoplasm constitutes a physical system characterized above all by its response*

[22] Cf. Biondi und Neumann, Die Fetteilschen im. Blut, Wiener Klin. Wech. 1910, Nr. 20.

[23] It is gratifying to note that Anitschkow has also been struck by the parallelism in the distribution of our vital stains and the fat bodies, and that he has adopted our explanation of the common physical cause. (Evans, Schulemann, Wilborn: Jahresb. d. Sch. ges. f. vat. Kul., 1913.)

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to finely particulate matter. In the case of particles of ordinary microscopic dimensions, this response (phagocytosis) is a behavior shared equally with the polymorphonuclear elements of the blood. But towards the very much finer[24] ultramicroscopic particles, the macrophages react in a practically specific way "drinking" them in, as it were, and storing them either as free coagula in their protoplasm or as the inhabitants of watery vacuoles where they oscillate in ceaseless Brownian movement.

From the definition of the macrophages which we are now able to formulate, their role in the body is foreshadowed. Little wonder that they should store substance of importance to the organism, for many of these are in the colloidal state. There is as little doubt but that their action in this capacity obeys the principle of a physiological balance, for only in cases in which the local or general content in the substance is very high do they load with it[25] and there is no question but that they liberate their content to an impoverished fluid. For the latter phenomenon no analogy could be more beautiful than that furnished by the decolorization of animals stained by these dyes, and the liberation of their dye content by the macrophages stands in direct relation again with the physical character of the dye solution, highly dispersed ones escaping rapidly, highly colloidal ones adhering stubbornly to their depots.

There can be little wonder that in all of those processes connected with tissue destruction the macrophages should house the complex chemical bodies set free and so become the great cells finally so evident to the eye, for there is growing recognition of the fact that many of these bodies are in colloidal systems like the dyes.

[24] This reaction is limited however to particles of certain dimensions for those vastly more minute and speedily diffusing particles of acid dyes which form true solutions, gain no admittance to the cell. By comparative measurements of the diffusion in gels, it is possible to recognize an intermediate group of dyes corresponding to a certain colloidal state which will act as typical stains. Using Wilborn's measurements we were able to predict the biological behavior of the dyes. See Evans and Schulemann, Die vitale Färbung mit sauren Farbstoffen, Deutsch. med. Woch. No. 30, 1914.

[25] Xanthelasmas are associated with diseases (e.g., diabetes and some of the icteric conditions) in which there is a general increase of cholesterin compounds. Pinkus u. Pick: Deut. med. Woch., 1908, v. 33.

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THE THRESHOLD STIMULUS OF THE CERVICAL SYMPATHETIC IN RELATION TO VASODILATION, VASOCONSTRICTION AND SALIVARY SECRETION

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In 1851 Bernard (1) noted in rabbits certain vasomotor effects upon cutting the cervical sympathetic nerve. In 1852 Brown-Séquard (2) and Bernard (1) and in 1853 Waller (3) observed constriction of the dilated vessels in the rabbit's ear upon stimulating the superior portion of the cut nerve. Since their discoveries other observers have added to the list of functions of this nerve e.g., salivary secretion, vasodilation in the submaxillary gland in cats, vasoconstriction in the nasal mucosa, retraction of the nictitating membrane, dilation of the pupil, erection of the hairs on the side of the face, dilation of the vessels of the eye, etc. In some cats Carlson (4) was able to produce vasodilation without salivary secretion and vice versa by means of stimuli of different strengths without however measuring the currents. In this connection he says:

> If the intensity of the interrupted current is carefully graduated up to the point where it just suffices to stimulate the vasodilator fibres, it is possible in some specimens to obtain marked vasodilation without any secretion from the gland. In some animals, however, I failed to secure this separation, as the minimal stimulus for the dilators was sufficient to stimulate the secretory fibres. In specimens giving the usual augmented blood flow on stimulation of the sympathetic with a weak interrupted current, the same results were usually obtained with currents of medium and of very great intensity, currents too strong to be applied to the tongue, but occasionally the very strong interrupted produced vasoconstriction (fig. 7 B).

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Mendenhall (5) recorded quite different threshold stimuli for vasoconstriction of the nasal mucosa, for dilation of the pupil, and for retraction of the nictitating membrane.

The thresholds for producing vasodilation and salivary secretion in the submaxillary gland in dogs upon stimulation of the chorda tympani nerve were determined to be the same (6). Two questions now arise: (1) Is there a difference, and if so how much, in the threshold stimuli of the chorda tympani and the cervical sympathetic nerves for the production of vasodilation and salivary secretion? (2) Does the cervical sympathetic nerve require stimuli of different strengths to produce these phenomena, and if so, what is the difference?

THE METHOD

In the earlier experiments cats were anaesthetized with ether and quickly decerebrated, but later urethane (2 grams per kilo body weight by stomach) was employed. The skin was incised on the median line of the neck and the mylo-hyoid muscle cut and laid back, exposing the submaxillary duct. A cannula narrowed at the distal end was inserted in Wharton's duct. By direct observation of the amount of fluid accumulating within this cannula even a very slight secretion of saliva could be detected. The left cervical sympathetic nerve was isolated and cut and a glass electrode placed on it so that the cathode was proximal to the gland (7).

Vasodilation and vasoconstriction were determined by the rate of blood-flow from the gland. A cannula was placed in the external jugular vein after all the veins contributing to it save the one leading from the submaxillary gland had been tied off. The rate of blood-flow was recorded by causing drops to fall upon a lever attached to a receiving tambour, which latter in turn was connected with a recording tambour provided with a lever to write upon the kymograph surface.

The threshold stimuli were measured by means of the Martin (8) method in which the strength of stimulus is calculated in β units. The strength of the primary current for these deter-

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minations was 0.1 ampere and the rate of stimulation was six or seven per second.

In all the experiments the readings for salivary secretion, (those for pupil dilation, and retraction of the nictitating membrane were made incidentally) were taken before the cannula was placed in the external jugular vein for use in determining vasomotor changes. In no case did the animal lose more than 25 to 35 cc. of blood before the completion of the experiment.

Since it was necessary to carry on this series of experiments upon cats it seemed advisable for purposes of comparison to make a series of readings upon these animals like those recently published upon the threshold stimuli of the chorda tympani in dogs. The method employed was the same as that used in the previous paper except the employment of electrodes like those used by Cannon and Nice (9) in stimulating the splanchnics. These were fastened on the isolated intact left chorda tympani nerve, care being taken to avoid drying or pulling of the nerve.

RESULTS

The threshold stimuli of the chorda tympani nerve producing salivary secretion and vasodilation in the submaxillary gland varied from 1.12 to 5.90, or an average for the fifteen experiments of 3.24 Z units. β units were also determined; and these varied from 0.68 to 2.76, or an average of 1.91 β units (see Table I). These are lower than the thresholds obtained for dogs—5.45 Z and 3.14 β units (6).

I found as did Mendenhall (5) that no two of the functions of the cervical sympathetic nerve were elicited by the same strength stimulus. Table II presents the results obtained upon decerebrate and Table III those upon urethanized cats.

The average threshold stimulus in Z and β units for decerebrate animals was found to be, for pupil dilation 2.99 Z and 2.09 β, for retraction of the nictitating membrane 4.15 Z and 2.95 β, for vasodilation 10.15 Z and 6.62 β, for vasoconstriction in the animals in which vasodilation was also recorded 11.43 Z and 7.51 β, for vasoconstriction for the whole series of decerebrate

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TABLE I

The threshold stimulus of the chorda tympani nerve in cats as shown by the dilation of the vessels in the submaxillary gland and by the secretion of saliva

Z UNITS	β UNITS	RATIO OF β TO Z
1.12	0.68	0.61
1.76	1.55	0.88
1.78	1.24	0.70
2.24	1.45	0.65
2.45	1.25	0.51
2.65	2.24	0.85
2.96	1.90	0.64
2.96	1.90	0.64
2.96	2.27	0.77
3.27	2.36	0.72
4.28	2.60	0.61
4.70	1.85	0.39
4.70	2.76	0.59
4.90	2.27	0.46
5.80	2.39	0.41
Average 3.24	1.91	0.63

Ratio of average β to average Z 0.59.

TABLE II

The threshold stimuli of certain functions of the cervical sympathetic nerve in decebrate cats

SALIVARY SECRETION		VASO-DILATION		VASO-CONSTRICTION		PUPIL DILATION		RETRACTION OF THE NICTITATING MEMBRANE	
Z	β	Z	β	Z	β	Z	β	Z	β
6.70	5.45	6.65	5.40	5.82	4.73	3.10	2.50	3.82	3.14
8.15	5.05	5.10	3.15	6.62	4.02	2.02	1.25	2.14	1.32
9.80	7.50	7.50	5.70	7.50	5.70	2.24	1.70	4.69	3.56
14.30	11.60	7.65	6.20	8.67	7.50	2.26	1.84	3.57	2.90
20.70	9.40	17.10	7.70	17.10	7.70	2.44	1.10	2.44	1.10
24.00	18.70	12.80	10.00	17.20	13.50	4.28	3.35	6.65	5.20
43.50	25.00	14.30	8.25	17.10	9.85	2.45	1.41	3.57	2.02
*14.30	10.70	—	—	7.65	5.25	3.88	2.91	5.80	4.35
*22.05	14.00	—	—	10.00	6.40	4.27	2.74	4.70	3.00
Average 18.17	11.93	10.15	6.62	10.85	7.18	2.99	2.09	4.15	2.95
Ratio β to Z	0.65		0.65		0.66		0.70		0.71

*Vasodilation was not obtained in these animals.

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animals 10.85 Z and 7.18 β, and for salivary secretion 18.17 Z and 11.93 β units.

The average threshold stimulus in urethanized animals was for pupil dilation 3.61 Z and 2.46 β, for retraction of the nictitating membrane 5.19 Z and 3.52 β, for vasodilation 7.10 Z and 5.33 β, for vasoconstriction 10.22 Z and 6.71 β, for vasoconstriction in which vasodilation was also recorded, 8.64 Z and 6.44 β, and for salivary secretion 22.58 Z and 14.28 β units.

TABLE III

The threshold stimuli of certain functions of the cervical sympathetic nerve in urethanized cats

SALIVARY SECRETION		VASO-DILATION		VASO-CONSTRICTION		PUPIL DILATION		RETRACTION OF THE NICTITATING MEMBRANE	
Z	β	Z	β	Z	β	Z	β	Z	β
10.00	7.60	5.80	4.40	8.67	6.60	2.24	1.70	2.96	2.25
14.30	10.90	5.10	3.90	3.57	2.74	2.35	1.80	2.96	2.26
29.30	21.40	10.40	7.70	13.50	10.00	3.65	2.70	6.37	4.70
*13.00	9.05			10.00	6.95	4.28	2.97	4.28	2.97
*15.65	8.00			8.77	4.50	3.50	2.44	5.02	3.50
*17.10	7.15			6.65	4.35	4.17	2.66	5.10	3.32
*21.70	14.30			8.67	5.74	4.27	2.83	6.62	4.38
*32.00	22.50			11.70	8.25	3.88	2.73	5.80	4.07
*50.20	27.60			20.50	11.30	4.17	2.30	7.62	4.20
Average 22.58	14.28	7.10	5.33	10.22	6.71	3.61	2.46	5.19	3.52
Ratio β to Z	0.63		0.75		0.66		0.68		0.68

*Vasodilation was not obtained in these animals.

The average thresholds for both decerebrate and urethanized animals was, for pupil dilation 3.30 Z and 2.27 β, for retraction of the nictitating membrane 4.67 Z and 3.23 β, for vasodilation 9.24 Z and 6.24 β, for vasoconstriction 10.53 Z and 6.95 β, for salivary secretion 20.37 Z and 13.10 β units.

The ratios of β to Z for the various functions of the cervical sympathetic varied from 0.63 to 0.75 and for the chorda tympani the ratio was 0.63 (see Tables I, II and III).

As the above figures show there is a great diversity between the thresholds of the various functions of the cervical sym-

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pathetic nerve e.g., the threshold for pupil dilation is 2.27 β units and that for salivary secretion almost six times as great, 13.10 β units. The results here obtained for pupil dilation and retraction of the nictitating membrane respectively (2.27 and 3.23 β units) are slightly lower than those recorded by Mendenhall (3.22 and 3.68 β units) (5). In the main these results corroborate Mendenhall's in that the pupil dilator threshold is the lower. The threshold stimuli of the chorda tympani and the cervical sympathetic supplying the same gland and producing the same changes within the gland differ remarkably. A current having the strength of 1.91 β units applied to the chorda tympani is sufficient to produce both vasodilation and salivary secretion in the submaxillary gland. To bring about these same changes upon stimulation of the cervical sympathetic 6.24 and 13.10 β units respectively are required.

Why such a difference in the thresholds of the functions of the same nerve should exist is still unknown. Various conditions working together or separately may be in part responsible, e.g., (1) there may be a difference in the character of the tissues supplied, (2) the nerve endings may be different in character some requiring a greater stimulus than others to produce the necessary changes, (3) the fibres for the different functions may possibly be located in groups within the nerve trunk and the stimulus may reach the axial fibres with greater difficulty than the surrounding fibres, making a stronger stimulus necessary, (4) if the number of fibres varies with each function of the nerve the strength of stimulus may vary. Langley (10) says, "The cranial nerve contains many, the sympathetic nerve comparatively few, secretory fibres."

The effect of anaesthesia. Whether urethane has or has not a depressant action upon all tissues is a disputed question. Dixon and Ranson (11) used urethane as an anaesthetic ($1\frac{1}{2}$ grams per kilo body weight injected intraperitoneally) in studying the broncho-dilator nerves in rabbits, and in their paper state that "It is well to remember that urethane has a depressant effect on all forms of plain muscle, and an excessive dose may easily invalidate an experiment." The writer (12) has shown

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that the threshold of a simple nerve muscle preparation may be increased almost three-fold, and that of the muscle almost two-fold, by the use of urethane as an anaesthetic. A two-fold increase in the threshold was observed by Martin and Lacy (13) upon the reflex-pressure-lowering mechanism. Without citing evidence to substantiate his claim, Nice (14) asserts that the threshold value of the phrenic-diaphragm preparation was the same in decerebrate and urethane animals. Recently Mendenhall (5) showed three examples taken from a long series of experiments upon urethanized, decerebrate, and etherized cats. The first two showed no difference in their effects, but the last required the use of a stimulus more than eight times as strong to bring about the same results.

As can be seen by studying Tables II and III in this article, a slight increase in the thresholds for pupil dilation, retraction of the nictitating membrane, and salivary secretion, slightly lower thresholds for vasodilation and vasoconstriction were obtained by the use of urethane. The lowered threshold for vasodilation is probably due to the fact that in only three cases out of nine were such readings made, and two of these animals had unusually low thresholds for all functions of the nerve.

In some animals only a decrease in blood-flow was obtained, in others this was followed by a marked augmentation upon cessation of stimulation. In other animals vasoconstriction or vasodilation occurred without after effects. In other animals there was no change in blood-flow during stimulation but a marked augmentation upon cessation of stimulation. In no case in which augmentation occurred during stimulation did a decrease occur upon cessation of stimulation.

In ten out of eighteen experiments vasodilation was obtained. Invariably strong currents produced vasoconstriction —never vasodilation as found by Carlson (4).

SUMMARY

1. The threshold stimuli of the cervical sympathetic in cats for vasodilation and salivary secretion (6.24 and 13.10 β units,

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respectively) are greater than the threshold stimulus of the chorda tympani for the same phenomena (1.91 β units each).

2. The threshold stimuli for the various functions of the cervical sympathetic nerve are different. They are, for pupil dilation 3.30 Z and 2.27 β units, for retraction of the nictitating membrane 4.67 Z and 3.23 β units, for vasodilation 9.24 Z and 6.24 β units, for vasoconstriction 10.53 Z and 6.95 β units, and for salivary secretion 20.37 Z and 13.10 β units.

3. The results here obtained for pupil dilation and retraction of the nictitating membrane confirm those of Mendenhall.

4. Carlson's observation that the cervical sympathetic in the cat contains both vasodilator and vasoconstrictor fibres to the submaxillary gland is confirmed.

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II. FURTHER STUDIES ON INTESTINAL RHYTHM

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In a recent paper (1) I have shown that segments of the rabbit's small intestine, beating in warm oxygenated Ringer's solution, have a much more rapid rhythm when taken from the duodenum than when taken from the lower ileum. Expressed roughly, the rate varies inversely as the distance from the pylorus. When the rates in different regions were charted as in figure 1; '*C*,' they were found to follow a fairly straight line from 15.5 waves per minute in the duodenum to 10 per minute near the ileo-cecal sphincter. Similar differences in rate were found also in the small intestine of animals opened under warm salt solution.

Other differences appeared when records were obtained from both ends of short excised loops, (7–12 cm. long). For instance, the oral end had a more regular rhythm and amplitude of contraction than the aboral end. The upper end of a loop or a segment from the upper intestine was also less affected by adrenalin, and recovered more rapidly from the inhibitory influence, than did a lower region. The more rapid rhythm and smaller amplitude of contraction in the duodenal segments was apparently associated with a greater tone of this region as compared with the lower ileum. The duodenal region was also found to be more irritable to mechanical and thermal stimulation than lower parts of the small intestine.

HISTORICAL

In May 1913 Dr. Cannon called my attention to the possible significance of the changes of rhythm which appeared in my tracings from excised segments of intestine. He had noticed

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such differences of rhythm while at work on other problems but he had never had time to look into the subject and did not remember having read anything about it.

During the next year a search was made through all the accessible recent literature: the work of Bayliss and Starling; Magnus and his pupils; the pharmacological studies in which intestinal strips were used; articles on the intestine in recent textbooks of physiology, etc., and little was found that would lead anyone to suspect that the neuromuscular tube of the small intestine is any different—anatomically or physiologically—in the duodenum from what it is in the ileum. Even Roith (2) who believes the peculiarities of colonic activity are based upon regional differences in musculature and tone, states that the small intestine is the same throughout.

Recently I obtained a copy of the English translation of Luciani's Textbook of Physiology (3) and there found that such differences had been observed. In speaking of some experiments to determine the speed and force of peristalsis in men and dogs with intestinal fistulae, he remarks that although the results were very variable, as should be expected from dissimilar strengths of the several parts of the gut, etc.; one important fact emerges, which harmonizes with the histological data, and that is: "that both rate and force of the intestinal movements diminish regularly from duodenum to ileum." He gives the rhythm of the duodenum and jejunum (of the rabbit?) as 14 to 23; that of the ileum as 12 to 18 per minute. I cannot find whether these conclusions are based on previously published work by Dr. Luciani or upon the results of others.

A renewed search through the older literature has yielded several references. Legros and Onimus (4) note that they "have found sometimes 18 contractions per minute in the duodenum of the dog and never more than 11 or 12 near the cecum. This peculiarity may help explain why the upper parts of the digestive canal are more often empty." They passed balloons into the duodenum from a gastric fistula and allowed them to travel down the intestine. A similar technic was followed by Hess (5) who quotes Nothnagel to the effect that the peristaltic waves

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in the upper portion of the bowel are more frequent and stronger than in the lower parts; and that they become less frequent the farther we go from the pylorus. This is apparently amplified from Nothnagel's statement (6) that in rabbits, it is known that the uppermost parts of the small intestine, particularly the duodenum, "starker und mehr als alle ubrigen Darmstrecken bewegt." Lüderitz (7) gives the rhythm in rabbits as 19–22 in the duodenum and 12–18 in the rest of the small intestine and colon. Pohl (8) quotes Lüderitz's findings as to rhythm without much comment.

It is of interest also to note that Stiles (9) has shown that the rhythm of circular strips of the frog's esophagus varies inversely as the distance from the pharyngeal end. Bottazzi (10) ascribed the aboral course of the waves in the esophagus of Aplysia to the greater tone and excitability of the oral region in which they arise. He suspected a difference in rate but he could not show it.

It seems strange that findings as definite as those of Legros and Onimus and Lüderitz should have been so completely forgotten. The Italian work could more easily be overlooked on account of its relative inaccessibility. The unsatisfactory state of our knowledge of intestinal peristalsis is due largely to the fact that most of those who have written on the subject were so interested in the problems of innervation and toxicology that they have paid scant attention to the normal progress of food through the tract.

The present paper embodies some of the results obtained from the study of the intact intestine with a new type of enterograph.

TECHNIC

The animals (rabbits unless otherwise stated) were kept under urethane (2 grams by stomach per kilo of body weight); and the spinal cord was destroyed as far up as the intrascapular region. The bath of salt solution was kept at 37°C. throughout the work.

The rhythm was counted with a stop-watch at first but it was apparent that no satisfactory work could be done without

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graphic records. The slow progress of our knowledge in regard to the motor functions of the small intestine and to the effect of purgatives, etc., has been due largely to the lack of simple recorders, a series of which could be attached so easily and lightly along the course of the intestine as not to interfere materially with its function.

The usual small balloon inserted through a hole in the gut is very undesirable—it is a foreign body blocking the lumen of the

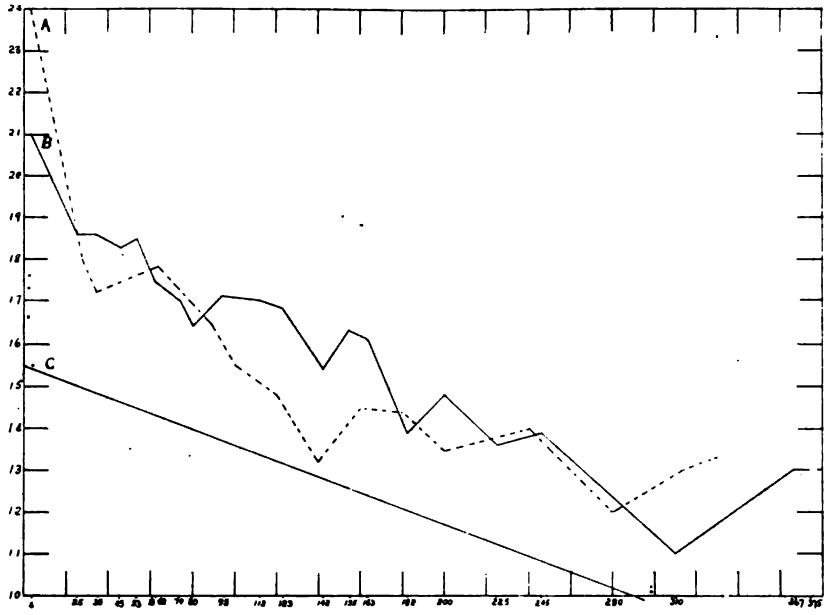


Fig. 1. Rates of rhythm in different parts of the small intestine of the rabbit. Ordinates represent rates per minute and the abscissae are distances in centimeters from the pylorus. Temperature: 37°C. Line *C* was obtained from excised segments; *A* and *B* from the intact animal. *A* represents the average in a single animal during active digestion. *B* is made up from 723 records in nearly 30 animals.

intestine, and the trauma incident to its introduction overshadows any other stimuli that it may be expected to record. Of all the methods for recording intestinal contractions mentioned in Tigerstedt's Handb. der physiol. Methodik (11), the one that comes nearest to the requirements is the enterograph of Bayliss and Starling (12). It is, however, rather too elaborate; it is not

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easily fastened to the bowel wall; it must be supported above the level of the salt solution and they admit that they could not use it on the more delicate and sensitive wall of the rabbit's intestine (13).

With the kind assistance of Dr. Saxton T. Pope, I have devised a recorder which is simple, cheap and easily attached to the gut. Figure 2 shows the shears made from two small pieces of aluminum. The rubber teat employed is one used to spring the shutter in large cameras and can be secured at photographic supply houses. At the end of the long arms are firmly attached tiny wire serrefines, which account for the ease of attachment to the peritoneal coat of the gut. This facility in attaching the recorders is essential because the tone of the bowel changes markedly from time to time. An actively digesting segment 3 cm. long may relax later, when the contents move onward, and leave a loop 8 cm. long. A temporary increase of tone at first, due to the stimulus of attachment, also tends to leave a slack loop later; and this must be taken up if a record is to be obtained. The distance between the serrefines is about 3 cm., but smaller sizes may be made. The leverage sacrifices amplitude to sensitiveness but this may be compensated for at the tambours. Contractions will appear on the drum that are barely noticeable on the bowel, and a hand-lens will sometimes show waves in an apparently straight record.

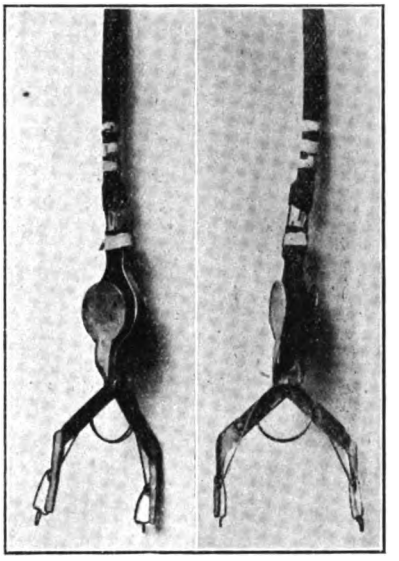


Fig. 2. Intestinal recorder described in the text. The three white bands on the tubing show that this instrument is connected with the third tambour at the kymograph.

Most of the work has been done with the simplest form of the apparatus. The light German silver spring, the stop to keep

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it from opening too far and the arm to keep the teat from working back and forth and cracking the cement which holds it to the aluminum are recent modifications still under trial. This arm must not bind or it will impair the sensitiveness of the apparatus. For the same reason the teat must not be too firmly and extensively cemented to the metal.

I now use seven recorders at a time; they seldom interfere with one another and their number is limited only by the width of the kymograph drum. A great advantage is that they give a continuous record of the activities of parts, such as the duodenojejunal junction, that lie hidden under other coils. They work splendidly in the rabbit; but may give some trouble in the cat and dog, whose intestinal contractions are so powerful that the muscle sometimes tears away, leaving the recorders hanging by threads of peritoneum. This can be obviated by sharpening and bending the points of the serrefines so that they will grip a little of the muscular coat.

The bowel is so very sensitive that it is doubtful if anything can cling to its walls without disturbing its functions to some degree. The slight stretching between the levers tends to keep the segments more active than they otherwise might be (14); but the fact that even the sensitive duodenal loop will remain inactive at times with four or five recorders on it, shows that the disturbance must be slight. A number of observations on the same region of bowel first with a stop-watch and later with recorders showed little difference in the rhythm. According to Bayliss and Starling's experience with balloons (15), changes in tension between the two serrefines are not likely to materially alter the rhythm. The possible objection that the apparatus records the contractions of the longitudinal muscle only, is lessened by the same authors' observation that the two layers ordinarily beat isorhythmically.

This apparatus is convenient also for studying excised segments of any tubular organ. They may first be attached gently so that the pieces can be snipped out and dropped into oxygenated Ringer's solution without further handling. In this way, it is easy to get simultaneous records from a number of excised segments.

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CURVES OF RHYTHM

Conclusions in this paper are based on records obtained from 30 rabbits. Only a few cats and dogs have been studied but these showed the same variations of rhythm as in the rabbit. After the recorders are attached, a continuous record is obtained of

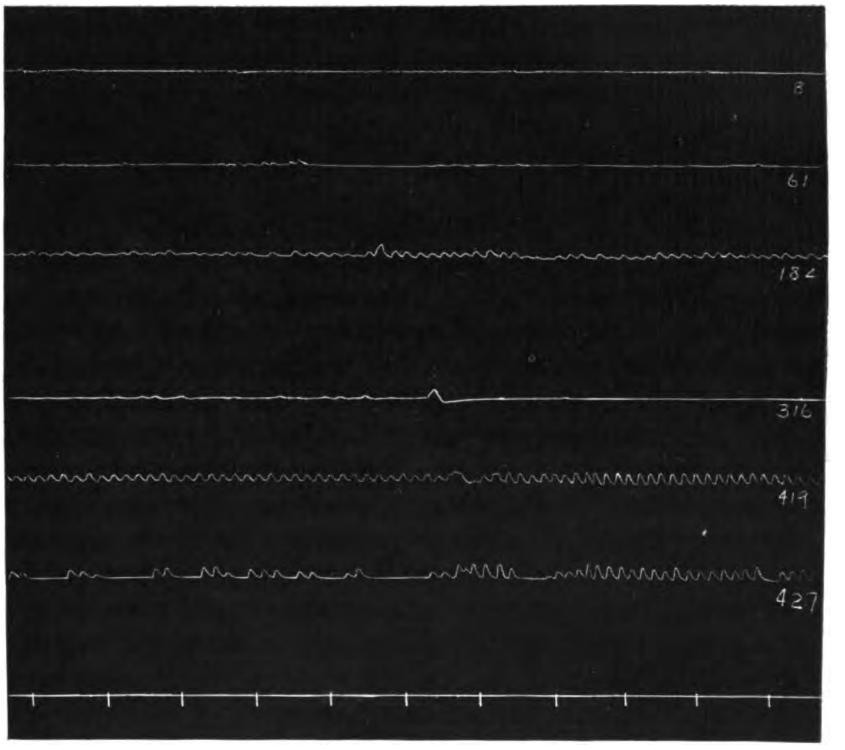


Fig. 3. A sample tracing from six recorders on a rabbit's small intestine. As previously mentioned, the amplitude of contractions is small in the duodenum. A diastalic wave may be observed in its progress from one end of the small intestine to the other. Time markings show 30 second intervals. The figures at the right represent distances from the pylorus, in centimeters.

the activities for about three hours. See figure 3. The rhythm is then counted for one-minute intervals every ten minutes or oftener; and the readings are charted on cross-section paper as in figures 4 and 5. The lines connect simultaneous readings.

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Line *A* in figure 1 is an average from all the readings in one animal; while *B* is derived in the same way from 723 readings in a large number of animals. *C* is taken from figure 3 in the previous paper; and was derived from the rhythms of *excised* segments. The general parallelism of *B* and *C* is apparent. The higher rate in *B* may be partly due to the higher temperature of the mesenterial blood supply, probably over 38°C., while the excised segments were in a bath at 37°C. The normal circulation must also be more efficient than an artificial makeshift and this might result in a faster rhythm.

DIFFERENCES BETWEEN CURVES *C* AND *B*

There are two marked deviations from the parallelism of the two lines. One is the rapid fall in the first part of the duodenum due to the disproportionally high rhythm near the pylorus; the other is the rise in the terminal ileum. These irregularities at the two ends of the small intestine were noticeable to a lesser degree with excised segments also; and it was shown that the last few centimeters of the ileum may receive a higher tone and rhythm from the muscular sacculus rotundus, which surrounds the ileo-cecal valve of the rabbit. There seems little doubt now that this is the case. One rabbit, whose ileum beat at times as fast as 18 per minute, had an unusually active cecum and colon; and when they were quiet, the rate generally dropped to 12–15 per minute. In a dog, large tonus waves appeared in the tracings from the ileum when the colon was active.

Similarly, the stomach was very active in all but one of the animals with a duodenal rhythm of 24 or 25 per minute; and in some of these, the rate dropped to 21 when the stomach was quiet. In another animal, the tracing from the duodenum, 25 cm. from the pylorus, showed a plateau of higher tone for eight minutes during a period of gastric activity. Figure 4 shows the steepness of the gradient in the first part of the curves from animals whose stomachs were active. The spread of tone from the cecum to the ileum and from the powerful pyloric portion of the stomach to the duodenum is in accord with v. Uexküll's

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(16) law that in a nerve net, such as Auerbach's plexus, the tonus will flow from higher to lower levels.

Figure 4 shows another peculiarity of the curve in the intact animal: the horizontal or slightly ascending line between 36 and 65 cm. This is apparent in the curve from the duodeno-jejunal region in almost every animal. It should be noted here that the duodenal loop in the rabbit is particularly long and free; and extends to a point, on an average, 60 cm. from the pylorus where it is closely attached to the spine. The comparatively level place in the curves always extends from about 20 cm. above to a few centimeters below this bend. This feature has been largely obliterated in the composite curve because the "Bend" varies in its location between 45 and 75 cm. from the pylorus.

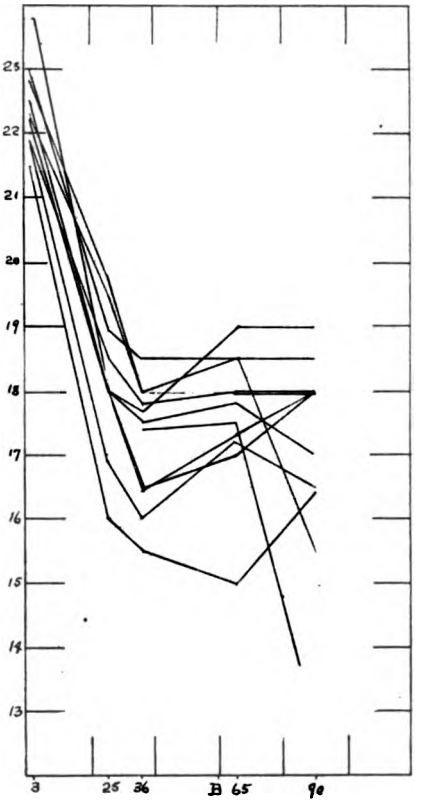


Fig. 4. Curves of rhythm in the duodenum of a rabbit whose stomach was very active. Ordinates represent rates per minute and abscissae distances in centimeters from the pylorus. *B* marks the duodeno-jejunal bend. Note the steep gradient at first and the slight rise to just beyond the "Bend."

What causes this peculiarity in the curve from the upper jejunum? The fact that similar rises in the duodenum and terminal ileum can be attributed to a spreading of tone from stomach and cecum suggested that, here also, there might be some outside source of tone. If this surmise be correct, a possible explanation for the curves may be found in the particularly rich and direct vagus supply to this region.

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VAGUS ENDINGS IN THE JEJUNUM

It has been shown repeatedly that vagus influence must reach the small intestine by way of the mesentery and not by extension from the stomach, as v. Braam Houckgeest (17) thought, because the effects persist after ligating the duodenum. See Mayer (18) and Jacobj (19). Apparently only the first part of the duodenum receives vagus branches from the stomach as Bayliss and Starling (20) found it more immediately and intimately under the control of that nerve. The second and third parts reacted like the rest of the small intestine. Miss Naylor, in making some dissections for them on three dogs, found (p. 140) one branch of the right vagus which, "after crossing the ganglion; proceeds alone between the layers of mesentery, not forming a plexus or running on an artery, and enters the wall of the intestine in the neighbourhood of the flexura duodeno-jejunalis." Figure 30 shows it just beyond this point.

Dr. R. W. Harvey of the Anatomical Department of the University of California made some dissections of rabbits to see if such an arrangement was present and kindly permits me to state that he found the distinct branch, separate from the plexus, running to the region of the duodeno-jejunal flexure. Recently I find that Jacobj (21) has described such direct branches of the vagus in rabbits. Remak (22) traced them in dogs, cats and in an infant. He says they are small and very numerous. Laignel-Lavastine (23) in his exhaustive study of the solar plexus, showed that the right vagus in man divides into three branches, two of which go to the semilunar ganglia, while the third goes to the intestine by way of a plexus on the superior mesenteric artery.

Edgeworth (24) showed that even some of the vagus branches that seem to end in ganglia appear intact on the other side without any interruption or connection with nerve cells. He was able to follow the large vagus fibers through serial sections of the superior mesenteric ganglia in the dog.

Further work should be done here as it seems more than a coincidence that this region, apparently in such direct communication with the brain, should be involved so often in strange and sometimes inexplicable surgical accidents.

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INTESTINAL RHYTHM IN THE CAT, DOG AND MAN

It was rather surprising to find the rates in the various parts of the small intestine of dogs and cats practically the same as those of corresponding regions in the rabbit. It seems probable also that there are similar differences in rhythm in the human intestine. The X-ray has cast little light upon the subject, largely on account of the difficulty in following the bismuth through the greater part of the small bowel. Kaestle and Bruegel (25), after making serial radiographic studies, state that they found little difference between the activities of jejunum and ileum except that progress in the former reached over greater stretches at a time, and *the kneading movements may there have been of a shorter duration.*

I have recently secured records from the human intestine by means of a balloon introduced through a fistula in the first part of the jejunum. Just below the fistula the rhythm varied from 10 to 19 per minute; two meters below it ranged between 8 and 11 per minute. The greater irregularity of the rhythm and the marked and sudden tone changes which appear in the first part of the jejunum make the gradation much less apparent than it is in the laboratory animals.

VARIATIONS DURING ACTIVE DIGESTION

As might be expected, the widest variations in tone and rate were seen in animals whose tracts were actively digesting. On opening the abdomen the intestine is often completely quiet, and the curve of rhythm from duodenum to cecum may be almost a straight line. Later, when the stomach begins to contract and food starts down the bowel, a series of irregular curves will be obtained as in figure 5. It appears from a number of such charts, that an active stomach is associated with a steeper gradient through the upper half or two-thirds of the small intestine, not only on account of a rise in the rate of the upper duodenum, but because of a fall in that of the ileum.

Animals with diarrhoea generally showed atypical, erratic curves which varied markedly from minute to minute.

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RAPID EMPTYING OF DUODENUM AND JEJUNUM

In a previous paper it was suggested that the more rapid rhythm in the duodenum and jejunum might have something to do with the well-known more rapid progress of food through that region. In the rabbit, material is churned back and forth between the two ends of a loop until the upper end overcomes the lower and a rush starts aborally. Other things being equal, it seems reasonable to suppose that if the oral end of a loop sends off 16 waves to the aboral end's 15, he balance will be upset finally in its favor and the rush will proceed aborally.

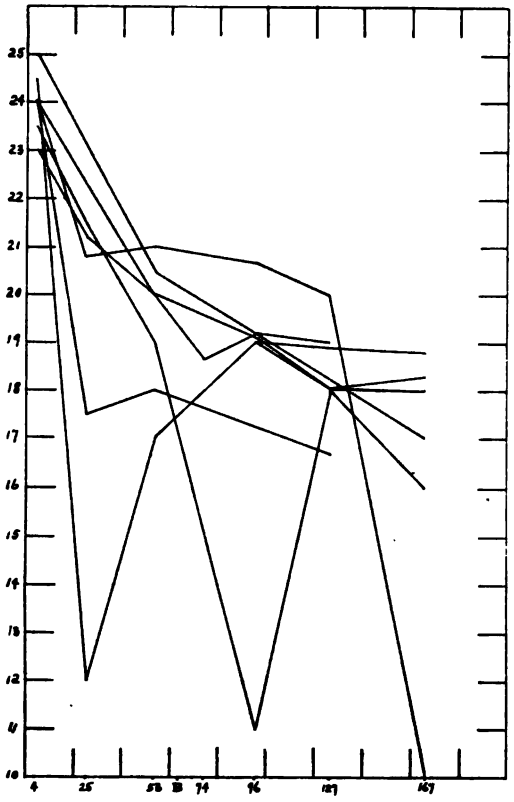


Fig. 5. Curves of rhythm in the duodenum and jejunum of an animal whose stomach was very active. Ordinates represent rates per minute and abscissae are distances in centimeters from the pylorus. Note the very steep gradients from time to time.

By attaching a recorder at each end of such a loop I have obtained graphic records of this process. In figure 6, the solid line represents the rhythm of the upper end, the broken line that of the lower end. For some time the two ends of the loop maintained about the same rate. They then began to fluctuate back and forth, and a rush nearly started at 1.33. This did not oc-

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cur, however, until 1.36 when, for the first time, there was a difference of 1.6 waves per minute between the rhythms of the two ends.

A glance at curves *A* and *B* in figure 1 shows that a loop of duodenum should have a large difference in rate at the two ends to begin with, while in the ileum, the rhythm might even be a little more rapid at the aboral end. On this score alone, the duodenal loop should very promptly spill its contents down the intestine. This explanation seems attractive, but it must be remembered that all rushes probably do not start in this way; also that the changes in rhythm, may be only signs of other more fun-

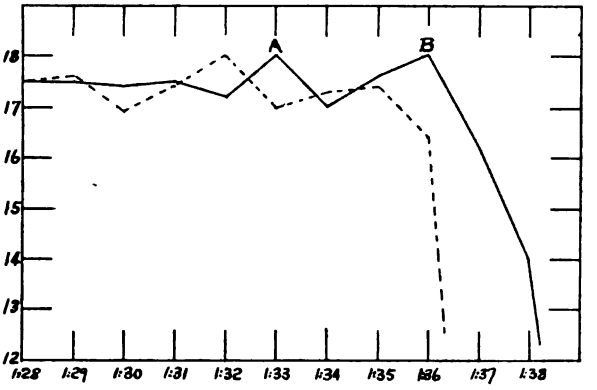


Fig. 6. Rates of rhythm at two ends of a short segment of intact duodenum. Ordinates are rates per minute; abscissae represent minutes of time. A rush started at *B* when the difference between the rhythm of the two ends was greatest. One nearly occurred at *A*.

damental processes taking place in the bowel wall. As I have intimated before, the rhythm may serve as an indicator of the varying degrees of muscular strength, of tone and excitability which are the real determiners of normal peristalsis. The contest between the two ends of the loop has been described in terms of rhythm only—other things, for the time, being assumed to be equal. In a subsequent paper I hope to show that they are far from equal; and the differences in muscular strength, in tone and irritability just touched upon in the previous article will be taken up in detail.

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I take pleasure in expressing my sense of indebtedness to Dr. Wallace I. Terry and Dr. Saxton T. Pope for many kindnesses and for their having extended to me the privileges of the University of California Laboratory.

SUMMARY

The rhythms obtained from excised segments of the rabbit's small bowel (beating in warm oxygenated Ringer's solution) and from corresponding regions of the *intact* intestine have been plotted side by side so that differences in the two curves are apparent. The relatively higher rate at the two ends of the intact intestine is probably due to a spread of tone from the stomach on the one side and the cecum and sacculus on the other. The slight rise in the first part of the jejunum may be due to the direct connection of this region with the vagus.

Similar curves have been obtained from the intestine of the cat and dog. There is also some evidence that the rhythm may be faster at the oral end of the human small intestine although the gradation is much less marked than it is in the laboratory animals.

The gradient of rhythm down the intestine is often steeper during digestion. It is suggested that the steep gradient through the duodenum and jejunum may have much to do with the more rapid progress of food through this part of the gut.

More important than the differences in rhythm are probably associated differences in muscular strength, tone and irritability, which will be discussed in more detail later.

The data used in this paper have been secured by means of a new enterograph, by means of which simultaneous records can be obtained from many parts of the intact intestine as it is exposed in a bath of warm salt solution.

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STUDIES ON THE PERMEABILITY OF THE INTERNAL CYTOPLASM OF ANIMAL AND PLANT CELLS[1]

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Some form of the plasma membrane theory of permeability is almost universally held at the present time. According to this conception the permeability relations and osmotic properties of cells are due to the presence of surface and vacuolar plasmatic membranes which are of unknown physical and chemical nature. The necessary corollary that the protoplasm proper be freely permeable to all classes of chemicals is generally accepted. Furthermore Pfeffer (1) was forced, by the result of his experiments on Hydrocharis, to assume that a plasmatic membrane is immediately formed on the freshly exposed surface of protoplasm when it comes in contact with water or other media. This view is based upon M. Traube's discovery and investigation of semipermeable precipitation membranes. Pfeffer in particular has shown remarkable ingenuity in the development of the plasma membrane theory but its foundations chiefly rest on indirect evidence, largely from analogy.

The literature is concerned for the most part with the kind of substances which may permeate into cells and the factors involved in permeability.

Overton (2), in his studies on the solubilities of dyes in mixtures of organic solvents and lipoids, assumed that the lipoid

[1] The studies on marine eggs reported in this paper were on during the summer of 1912 at the Marine Biological Laboratory, Woods Hole, Mass., while occupying a table through the courtesy of the Director, Dr. F. R. Lillie, to whom I am indebted for many kindnesses. An abstract of the observations was published in the Biological Bulletin, 1913, xxv, 1.

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and not the organic compound was solely responsible for the solution of the dye. The following example shows that this supposition is untenable. Thionin is insoluble in olive oil, benzol and olive oil-lecithin but it is quite soluble in benzol-lecithin. If lecithin were the only factor involved, as asserted by Overton, the thionin should go into solution in olive oil-lecithin. Moreover Loewe (3) found that lipoids do not form true solutions in the organic compounds which were employed by Overton and that dyes are adsorbed by lipoids when added to mixtures of lipoids and organic solvents.

Rhuland (4), Höber (5) are among those who have discussed in detail the factors which favor and prevent the entrance of substances into cells.

Recently Moore and Roaf (6), Meigs (7) and others have questioned the existence of plasmatic membranes; Harvey (8) has made refinements of the indicator method and Evans and Schuleman (9) have materially extended our conception of the factors involved in vital staining with the benzidine dyes. But if future investigations on permeability are to follow sound lines of advance the basic assumptions of the plasma membrane theory must be subjected to experimental study by direct methods. In this paper the results of such a study by means of new methods of technique which permit actual injection of dyes into living cells are given.

MATERIAL AND METHODS

One of the aims of this investigation has been to select species of animals and plants from widely different genera and phyla. The material comprised cells that show great structural and functional differences because it was thought that such cells might show special permeability relationships. The animal material included the eggs of Asterias, Cumingia, Chaetopterus, Nereis, the immature eggs of Necturus, Ameba proteus, Paramecium and striped muscle-cells and epidermal cells of Necturus. The plants used were Saccharomyces, Mucor, Saprolegnia, a number of species of Spyrogyra, Hydrodictyon, the manubrial

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cells of Chara, the leaves of Elodea, root-hairs of Vicia faba, Pisum and Hordeum, and the parenchyma cells of Tradescantia.

Barber's intracellular injection method was used for injecting dyes and crystalloids into the interior of cells. Punctures and dissections were made with extremely fine glass needles drawn on the end of Jena glass capillary tubes. For such operations, the cells were mounted in a hanging drop in an open-end moist chamber (10). In some experiments the permeability of the surface layer of marine eggs was modified by the application of very dilute acid, alkali or saponin. The volume of doses was calculated by measuring the diameter of the vacuoles produced. In staining by immersion, both basic and acid dyes were used in closely graded concentrations up to saturated solutions. The length of application was from a few minutes to several hours. In most cases dilute solutions were used, but many cells proved to be impermeable to long applications of saturated solutions of certain of the acid dyes. It may be emphasized that all the observations were made before any evidence of death changes could be detected.

The following dyes were employed. Unless stated to the contrary it is to be understood that they are manufactured by Grüber. A table of the more important solubilities is appended.

THE PERMEABILITY OF CELLS TO BASIC DYES

Janus green (Metz & Co.). (Obtained through the kindness of Prof. R. R. Bensley.) In dilute solution in sea water janus green quickly stained slate blue, granules, about a micron in diameter scattered throughout the cytoplasm of the egg of Asterias. A very small cytoplasmic granule in the egg of Nereis is vitally stained in a few minutes. The whole cytoplasm of the eggs of Cumingia and Chaetopterus was found to contain granules or globules of varying size, which may be stained beautifully by this dye. Granules and the whole cytoplasm of Mucor, Saprolegnia, Paramecium and Ameba are stained vitally by janus green.

New methylene blue G. G. A dilute solution of this dye in sea water stained vitally granules or globules, varying in size

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from about 2–10 microns, scattered throughout the cytoplasm of the eggs of Asterias, Cumingia, Chaetopterus and Nereis. The cytoplasm of Paramecium is stained quickly by a dilute solution of the dye in distilled water and the food vacuoles be-

TABLE 1

Basic dyes

	SOLUBLE IN OLIVE OIL	SOLUBLE IN BENZOL	SOLUBLE IN BENZOL-LECITHIN	SOLUBLE OLIVE OIL-LECITHIN
Methylene blue			Quite soluble	
New methylene blue N. (Cassella Color Co.)			Quite soluble	Trace in 24 hours
New methylene blue G. G. (Cassella Color Co.)	Very slightly soluble	Slightly soluble	Very soluble	
New methylene blue R. (Cassella Color Co.)	Very slightly soluble	Slightly soluble	Very soluble	
Toluidin blue	Trace soluble	Trace	Quite soluble	
Pyronin			Quite soluble	
Janus Green	Slight staining of surface		Quite soluble	Trace in 24 hours
Janus Green (Metz & Co.)	Slight staining of surface			Slightly soluble
Methyl Green				
Vesuvin	Slightly soluble		Soluble	
Neutral red	Stains surface	Trace	Quite soluble	
Thionin			Soluble	
Safranin (Water soluble)			Very soluble	Trace

come very distinct. The protoplasm of Mucor and Saprolegnia is beautifully stained vitally by a short application of a dilute solution of New methylene blue G. G.

New methylene blue R. Vital staining of various sized cytoplasmic granules of the marine eggs studied followed the application of a dilute solution of New methylene blue R, in sea water.

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TABLE 2

Acid dyes

	SOLUBLE IN OLIVE OIL	SOLUBLE IN OLIVE OIL-LECITHIN	SOLUBLE IN BENZOL
Orange G			
Bleu de Lyon			
Orcein			
Trypan blue			
Trypan red			
Isamin blue			
Eosin		Trace in 24 hours	
Erythrosin		Soluble in 24 hours	Deep staining in 24 hours
Ponceau P. R.			
Aniline blue			
Acid violet			
Nigrosin			
Indigo-carmine			
Biebricher scharlach			
Bordeaux R		Trace in 24 hours	
Aurantia		Trace in 24 hours	Slightly soluble in 24 hours
Indulin			Slightly soluble in 24 hours
Saüregrün			
Solid blue R (Cassella Color Co.)			
Thiocarmine R (Cassella Color Co.)		Soluble in 24 hours	
Acid fuchsin			
Methyl orange		Very slightly soluble	
Congo red			
Methyl red (Kahlbaum)	Soluble	Moderately soluble	
Azolitmin (Kahlbaum)		Trace soluble	
Tropeolin 000 No. 1 (Koenig & Co.)		Soluble	
Sodium alizarin sulphonate (Merck)		Slightly soluble	

The cytoplasm of Paramecium, Ameba proteus, Saprolegnia, Mucor and Spyrogyra was stained blue vitally. Such structural elements as granules and vacuoles were brought out sharply in the living cell.

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THE PERMEABILITY OF CELLS TO ACID DYES

Kuster (11) and Ruhland (4) have recently published accounts of many exceptions to Overton's conclusions regarding the impermeability of cells to acid dyes. By selecting species of animals and plants that have been neglected in most studies on permeability, I have been able to extend as follows, the list of cells permeable to acid dyes:

Trypan blue. The eggs of Nereis and Chaetopterus. The root-hairs of barley and the Windsor bean. Immature Necturus eggs. The peritoneal epithelium of Necturus. Ameba proteus, Paramecium, Mucor and Saprolegnia.

Trypan red. The eggs of Cumingia and Chaetopterus. The root-hairs of barley, the edible pea and the Windsor bean. Ameba proteus, Paramecium, Mucor and Saprolegnia.

Isamin blue. The root-hairs of barley and the Windsor bean.

Aniline blue. The eggs of Chaetopterus. The root-hairs of barley and the Windsor bean, and Mucor.

Acid Fuchsin. The root-hairs of barley and the Windsor bean, and Mucor.

Acid violet. Windsor bean root-hairs. Saprolegnia and Mucor.

Biebricher scarlach. Barley root-hairs. The immature eggs and the peritoneal epithelium of Necturus. Mucor, Saprolegnia and Paramecium.

Indigo-carmine. Saprolegnia and Mucor.

Ponceau P. R. The root-hairs of the Windsor bean. Ameba proteus, Paramecium, Saprolegnia and Mucor.

Nigrosin. Mucor. The peritoneal epithelium and the very immature eggs of Necturus.

Indulin. Root-hairs of Windsor bean. Ameba proteus, Saprolegnia and Mucor.

Eosin. Windsor bean root-hairs, Ameba proteus, Paramecium, Mucor and Saprolegnia.

Erythrosin. Mucor, Saprolegnia and the root-hairs of the edible pea, barley and the Windsor bean. Immature eggs of Necturus.

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Bordeaux, R. Paramecium, Mucor, Saprolegnia. The root-hairs of the edible pea and barley.

Aurantia. Mucor, Saprolegnia, Paramecium. The root-hairs of the Windsor bean and the immature eggs of Necturus.

Tropeolin 000 No. 1. Saprolegnia and Paramecium.

THE INTRACELLULAR INJECTION OF DYES

The permeability of the internal portions of the protoplasm to dyes was determined in the following manner. The cells were mounted in a hanging-drop in an open-end moist chamber and the injections were made with very fine capillary pipettes, according to Barber's method.

The eggs of Asterias. A large number of deep intracytoplasmic injections of indigo-carmine, thiocarmine R. trypan blue, methyl red and azolitmin were made. Saturated solutions of the dyes in sea water were used. Small doses varied between about 500 cubic microns and six times that amount.

The general result was the same in all cases. The solution of a given dye formed a cytoplasmic vacuole, out of which the sea water slowly diffused into the surrounding cytoplasm, leaving finally a small mass of precipitated dye to mark the position of the vacuole. In no case did the dye diffuse out of the vacuole into the surrounding cytoplasm. Even in the case of trypan blue, nothing more than an apparent light staining of the wall of the vacuole resulted and this was only obtained twice. The effect of a very slow continuous injection was tried. The capillary pipette was pushed deep into the cytoplasm and a very slow continuous injection made. By this means vacuoles of dye solution of all sizes up to one-fourth of the volume of the egg were produced but without diffusion of the dye into the cytoplasm. Rapid injections of large doses caused the egg to burst and sometimes to separate into several pieces. If the pieces did not undergo an appreciable swelling, no dye diffused into them.

The rate of diffusion of sea water out of cytoplasmic vacuoles is of general biological interest. A vacuole 9 microns in diameter,

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filled with a solution of trypan blue in sea water, disappeared in about fifteen minutes, while a vacuole of the same solution twenty microns in diameter required over sixty minutes for complete disappearance of its fluid content.

Ameba proteus. It was determined by experiment that the best results were to be obtained by dissolving the dyes used in about a $\frac{3}{4}$ molar cane sugar solution. Azolitmin, congo red, tropeolin 000, No. 1, sodium alizarin sulphonate, methyl orange, trypan blue and indigo-carmine dissolved in from $\frac{1}{2}$–1 molar cane sugar were injected into the endoplasm of Ameba. The vacuoles formed by the injected dye usually collapsed in a few seconds and the dye rapidly diffused through the endoplasm.

Very small doses of indigo-carmine dissolved in either distilled water or $\frac{1}{2}$ molar cane sugar were injected into the ectoplasm. Vacuoles resulted which usually broke into the endoplasm and disappeared in the course of ten minutes. Neither intraectoplasmic or endoplasmic injections resulted in staining of the ectoplasm.

Striped muscle-cell of Necturus. Indigo-carmine and trypan blue were selected for injection into the striped muscle-cells of Necturus. The dyes were dissolved in 0.8 per cent sodium chloride solution. Indigo-carmine remained entirely localized; trypan blue stained the granular wall of the vacuole and the immediately contiguous muscle substance.

Spyrogyra. A minute drop of a saturated solution of indigo-carmine in distilled water was injected into the sap vacuole of Spyrogyra. In ten minutes the whole protoplasm was blue-green in color. The same experiment was repeated using eosin and erythrosin. A few minutes after injection of either dye, the protoplasm became pink or light red. Vital staining of the cytoplasm and nucleus resulted from the intravacuolar injections of any of the common acid dyes which did not penetrate Spyrogyra when applied to the outer surface.

Some shrinkage of the cytoplasm usually resulted from the puncture of the cellulose wall and the acid dyes, unless they were very dilute, commonly caused the appearance of granules in the cytoplasm.

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Hydrodictyon. The whole protoplasm became stained ten to fifteen minutes after the intravacuolar injection of medium sized doses of saturated aqueous solutions of such dyes as indigo-carmine, erythrosin and trypan blue.

THE INTRACELLULAR INJECTION OF CRYSTALLOIDS AND WATER

The permeability of the internal parts of the protoplasm to crystalloids and water was studied by similar methods with the following results:

The eggs of Asterias. The walls of the vacuoles formed by the injection of distilled water and salt solutions were sometimes irregular but the measurements are of sufficient accuracy to be of considerable quantitative value. It was found that a small dose of distilled water diffused into the cytoplasm comparatively slowly. A dose of sea water of the same size required several times as long to disappear; while a vacuole of hypertonic sea water increased in size. It appeared from these results that any part of the cytoplasm might exhibit the same sort of osmotic properties as are shown by the surface. A mature starfish egg was mounted in a hanging-drop. 10,000 cubic microns of distilled water were injected deep into the cytoplasm. At the end of fifteen minutes the vacuole disappeared. Many repetitions of this experiment failed to show any important variations. A 7350 cubic micron dose of sea water was injected into the center of a mature egg. Forty-five minutes later it had disappeared. Repetitions of this experiment brought out no great variation. An 8440 cubic micron dose of slightly hypertonic sea water was injected into the cytoplasm of a starfish egg at 3.30 p.m. At 4.30 the volume was 9500. At 4.40 and at 5 it still remained about 9500 cubic microns in size.

Ameba proteus. Small and large doses of 1 molar sodium chloride, 1 molar potassium nitrate and from $\frac{1}{2}$ to 2 molar cane sugar solution were injected into the endoplasm of Ameba proteus. With the exception of the $1\frac{1}{2}$ molecular cane sugar solution, all the crystalloids injected produced vacuoles which usually collapsed in a few seconds, the vacuolar fluid dif-

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fusing through the endoplasm. An injection of very small doses of 0.9 per cent sodium chloride solution was made into the ectoplasm and well defined vacuoles were produced which broke into the endoplasm in the course of a few minutes. The ectoplasm was almost impermeable to a weak sodium chloride solution. Similar experiments were performed with distilled water. The distilled water formed a vacuole in the ectoplasm which showed no appreciable shrinkage in the five to eight minutes which usually intervened between the injection and the breaking of the vacuole into the endoplasm. Small doses of distilled water injected into the endoplasm did not form vacuoles. The water was immediately imbibed by the endoplasm. The injection of large doses, either gave a like result, or the ectoplasm was ruptured and the endoplasm imbibed water so rapidly that the cytoplasm immediately changed into the sol state.

The striped muscle-cell of Necturus. The muscle preparation was made by teasing. A dose of 1200 cubic microns of 9 per cent sodium chloride was injected deep into the sarcoplasm. In fifty minutes the vacuole had about disappeared. Another 1300 cubic micron dose of the same strength sodium chloride required over an hour to disappear. An injection of distilled water took about an hour to be imbibed by the surrounding sarcoplasm. Another very large dose of distilled water did not disappear in one hour and one-half.

When doses of from $1\frac{1}{2}$–2 molar cane sugar were injected the muscle died and became granular in the course of two hours; but little change in the size of the vacuole was observed except in one or two cases where there seemed to be a little increase in size. Exact quantitative measurements could not be made as the muscle-substance was so rigid that the vacuoles were very irregular in outline. As a result it was impossible to make exact measurements for the calculation of the volume of the vacuole. The measurements were exact enough to show that the sarcoplasm of the striped muscle-cell of Necturus is only slightly permeable to water, somwehat less so to 0.9 per cent sodium chloride and still less to strong cane sugar solution.

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THE EFFECT OF PUNCTURE ON THE PERMEABILITY OF MARINE EGGS FOR DYES

The eggs of Asterias, Cumingia, Chaetopterus and Nereis were selected for this series of experiments. The permeability of the normal uninjured eggs for concentrated solutions of acid dyes was determined, and only those dyes were used which did not stain the uninjured egg after application lasting from 30 minutes to several hours.

It was determined experimentally that slight incisions and punctures of the cytoplasm cause localized swellings of the nature of concentration gradients. The cytoplasm of such a swollen area increases gradually in concentration from the surface to the interior. A curve of the gradient would start at the surface which would represent the base line and slowly rise, then suddenly steepen and soon become flat. The flat top would represent the normal cytoplasm. The swollen area produced by cutting, or puncture, usually measured between 6 and 30 microns in thickness. The eggs were mounted in a hanging-drop in an open-end moist chamber. A Jena glass needle held in a three-movement pipette holder was used for incising the eggs.

Many variations of these experiments were made. The concentrations and the length of application of the dye and the size of the incisions and punctures were all varied. The dyes were dissolved in sea water. It was necessary in this kind of experiment to use dyes concentrated enough to be visible in small drops.

The purpose of the production of fluidity gradients in marine eggs was to determine whether their permeability relations are due to peculiar surface films, or to the whole protoplasm. If the current plasma-membrane theory of permeability be correct, a dye which penetrates the surfaces of a swollen area of cytoplasm ought to penetrate the whole interior of the egg.

In the majority of cases the dye did not penetrate the surface of the swollen area of cytoplasm. Those dyes which entered the surface of such swollen areas stopped at varying levels in the gradient. In no case was the whole interior of the egg stained.

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TABLE 3

Egg of Asterias

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Trypan blue	Moderately concentrated			
Trypan red	Moderately concentrated			
Solid blue R	Moderately concentrated	− (40m)	− (40m)	
Azolitmin	Moderately concentrated			
Nigrosin	Moderately concentrated			
Thiocarmine R	Moderately concentrated		+	
Tropeolin 000 No. 1	Moderately concentrated			
Blue de Lyon	Moderately concentrated		− (30m)	
Eosin	Dilute	+ (10m)	+ (10m)	
Erythrosin	Dilute	+	+	
Janus green (Grübler)	Dilute		+	

TABLE 4

Egg of Nereis

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Azolitmin	Concentrated	− (30m)		
Trypan red	Moderately concentrated	+	+	
Thiocarmine R	Dilute	− (30m)	− (30m)	

TABLE 5

Egg of Chaetopterus

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Solid blue R	Concentrated			
Erythrosin	Concentrated	+ (30m)	+ (30m)	
Eosin	Dilute			
Nigrosin	Concentrated			

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TABLE 6

Egg of Cumingia

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Trypan blue	Dilute			
	Concentrated	+	+	
Thiocarmine R.	Dilute	− (30m)	− (30m)	
Nigrosin	Dilute	+	+	
Solid blue R.	Dilute			
Erythrosin	Dilute	+	+	

THE EFFECT OF PUNCTURE OF THE CELL-WALL ON THE PERMEABILITY OF PLANT CELLS FOR ACID DYES

Puncture of the cell-walls of plants has given results that seem to be of signal importance for the general problem of permeability.

Hydrodictyon, Saprolegnia, some five species of Spyrogyra, the leaves of Elodea, the leaf parenchyma of Tradescantia and the manubrial cells of Chara were used in these experiments. The material was mounted in a hanging-drop. The cells walls of most of the species tested were extraordinarily tough and rigid so much so that the Jena glass needles were frequently broken in making the punctures. Both protoplast and cell-wall were punctured in many cases. The acid dyes employed were dissolved in either distilled water or tap water which was neutral to litmus but slightly alkaline to phenothalein. The solution of the dye was always concentrated enough to give a distinct color to microscopical droplets. The chief effect of increasing the concentration of a dye solution was to increase the speed and depth of staining. The dye was applied either before or after puncture of the cell-wall. Briefly stated, puncture of the cell-wall, or the cell-wall and protoplast, made most of the cells permeable to all the common acid dyes which under the same conditions do not penetrate the unpunctured cells. The protoplast was in some cases separated from the cell-wall by slight plasmolysis with about $\frac{1}{2}$ molar cane sugar and the cell-wall was then very carefully cut or punctured without in-

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juring the protoplast. In such cases the acid dyes penetrated the cytoplasm and nucleus about as readily as after puncture of the protoplast. If the cells were first thoroughly plasmolyzed and the walls cut acid dyes in quite concentrated solution occasionally stained the outer surface of the shrunken cytoplasm.

TABLE 7

Spyrogyra

DYE	SOLUTION	CELL WALL + PROTOPLAST PUNCTURED	CONTROL NOT PUNCTURED
Erythrosin	Moderately concentrated	+ (5 min.)	
Eosin	Dilute	+ (8 min.)	
Orange G	Moderately concentrated	+ (3 min.)	
Indigo-carmine	Concentrated	+ ((4 min.)	
Trypan blue	Moderately dilute	+ (10 min.)	
Trypan red	Moderately concentrated	+ (3 min.)	
Isamin blue	Saturated	+ (surface stained only) (10 min.)	
Aniline blue	Moderately concentrated	+ (8 min.)	
Nigrosin	Fairly concentrated	+ (10 min.)	
Thiocarmine	Fairly concentrated	+ (20 min.)	
Tropeolin 000 No. 1	Moderately concentrated	+ (10 min.)	(slight)
Methyl orange	Dilute	+ (10 min.)	(slight)
Methyl red	Moderately concentrated	+ (10 min.)	
Methylgrün	Slightly concentrated	+ (15 min.)	
Congo red	Moderately concentrated	+ (10 min.)	
Acid fuchsin	Moderately dilute	+ (4 min.)	
Biebricher Scharlach	Comparatively dilute	+ (5 min.)	
Bordeaux R	Moderately dilute	+ (4 min.)	
Pouceau P. R	Dilute	+ (5 min.)	

It was noted that rapid plasmolysis of spyrogyra is accompanied by a definite softening and loss of rigidity of the cell-wall. This fact was determined by dissecting plasmolyzed cells. Furthermore, it was determined that many acid dyes will enter and stain the whole protoplasm of Spyrogyra cells

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if added very soon after recovery from rapid plasmolysis. A mucilaginous substance usually poured out of the cell-wall of all the species of Spyrogyra examined during rapid plasmolysis. This substance can be stained red in neutral or very weakly alkaline solutions of sodium alizarin sulphonate.

Such facts prove conclusively that neither the plasma membrane nor the protoplast, but the cell-wall is responsible for the impermeability of many plant cells for acid dyes.

On account of the length of this series of experiments only selected examples can be given.

TABLE 8

Slightly plasmolysed Spyrogyra

DYE	SOLUTION	PLASMOLYSED WITH PUNCTURED CELL WALL. PLASMA MEMBRANE UNINJURED	CONTROL PLASMOLYSED WITH CELL WALL AND PLASMA MEMBRANE UNINJURED
Biebricher scharlach..	Very dilute	+ (8 min.)	
Bordeaux R.....	Very dilute	+ (2 min.)	+ (8 min.)
Erythrosin.....	Dilute	+ (5 min.)	+ (30 min.)
Ponceau.....	Dilute	+ (5 min.)	+ (30 min.)
Indigo-carmine.....	Dilute	+ (5 min.)	+ (15 min.)

TABLE 9

Plasmolysed Spyrogyra allowed to recover

DYE	SOLUTION	AFTER RECOVERY FROM PLASMOLYSIS	CONTROL NOT PLASMOLYZED
Biebricher scharlach.....	Moderately concentrated	+ (4 min.)	
Saure violet.....	Moderately concentrated	+ (3 min.)	
Bordeaux R.....	Moderately concentrated	+ (8 min.)	
Indigo-carmine.....	Moderately concentrated	+ (15 min.)	
Aniline blue.....	Moderately concentrated	+ (10 min.)	

THE EFFECT OF ACIDS, ALKALI, AND SAPONIN ON THE PERMEABILITY OF MARINE EGGS

It was determined by experiment that fluidity gradients could be produced in the cortex of marine eggs, by the application of very weak acids, alkali and saponin. The amount of swelling was controlled by direct observation. The eggs of Chaetop-

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terus, Cumingia, Nereis and Asterias were chosen for this experiment on account of their large size and favorable optical properties.

Unfertilized marine eggs which were allowed to lie in sea water, underwent cortical swelling in from six to thirty-six hours. Such changes are usually interpreted as autolytic. This is another method for establishing fluidity gradients in marine eggs. The results of all the methods were essentially the same. Acid dyes were selected which did not penetrate normal marine eggs even after long applications of concentrated solutions. The eggs were treated with very dilute acid, alkali, or saponin or allowed to autolyze until a definite cortical swelling occurred; then the eggs were washed in several changes of fresh water and solutions of the acid dyes added. Some of the dyes did not penetrate the surface of the swollen cortex; others entered to the depth from 3 to 8 microns; and still others as far as 15 microns, or the beginning of the unswollen cytoplasm. It is self evident that if a plasma-film prevents the entrance of these acid dyes and if the dyes once pass beyond the surface-film, the whole cytoplasm ought to be immediately stained; but the dyes are actually stopped at different levels of the gradient. An extract from my protocols will serve to make this clear.

Eight drops of 1 per cent solution of saponin in sea water were added to 20 cc. of sea water containing fresh Nereis eggs. In five minutes sufficient swelling occurred to establish the desired fluidity gradients in the cortex.

A five minute application of a moderately concentrated solution of erythrosin in sea water resulted in a pink to deep red staining of the cortex. The interior of the egg remained unstained twenty-five minutes later. The cortical swelling was commonly very unequal and some of the eggs showed general cytoplasmic swelling, being twice their normal diameter. Such eggs may become red throughout in thirteen minutes.

Nigrosin. A moderately concentrated solution of nigrosin in twenty minutes only stained the surface of eggs showing very definite cortical swellings.

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A fairly concentrated solution of trypan red only stained the surface of the cytoplasm at the end of twenty minutes.

My protocols contain the details of such experiments on the eggs of Chaetopterus Nereis, Asterias, and Cumingia carried out with a long series of acid dyes and using acid, alkali, saponin and autolysis for the production of fluidity gradients in the cortex. The results have been stated.

THE DISSECTION OF CELLS

The physical state of the structural components of many of the cells used in this investigation was determined by an adequate method of cellular dissection (10). The facts derived by this method are a part of the basis for my conclusions.

CONCLUSIONS

In previous studies on permeability the interior portions of cells have been assumed to be freely permeable to dyes and crystalloids the whole emphasis being placed on the properties of a hypothetical plasmatic membrane which is believed to be fundamentally different from the deeper parts of the cells. Now the new methods of approach employed in this work permitted of a study of the permeability of the interior portions of living cells. The observations on the staining of incised and punctured marine eggs, embodied in tables 3, 4, 5 and 6, have shown very clearly that the whole protoplasm offers a barrier against the penetration of dyes. The experiments with spyrogyra (tables 7, 8 and 9) have brought to light the fact that the internal protoplasm of plant cells is in reality freely permeable to a large variety of dyes when the obstacle formed by the cellulose membrane is removed. These and the other observations which have been recorded in detail are evidently incompatible with the plasma membrane theory as usually stated.

1. The structural components of protoplasm vary greatly in their permeability to water, dyes and crystalloids.

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2. Impermeability or partial permeability to water, dyes and crystalloids is a property of all portions of protoplasmic gels.

3. The rate of penetration of protoplasm by dyes and crystalloids is, in general, inversely proportional to the concentration of the living gel.

4. The best vital stains known penetrate such highly concentrated protoplasm as the epithelial and striped muscle cells of Necturus very slowly.

5. The interior portions of the cytoplasm of the starfish egg and probably the striped muscle cell of Necturus exhibit the same sort of osmotic properties as the surface.

6. The cell-walls and not the protoplasm of many plant cells prevent the entrance of dyes.

7. A study of the penetration by dyes of concentration gradients in living matter has been made.

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THE INFLUENCE OF EYE-MOVEMENTS IN JUDGMENTS OF NUMBER

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Into one's estimate of any indeterminate number of objects, such as a crowd on the street or a handful of cherries in a dish, a variety of factors doubtlessly enters. The space over which the objects are scattered, the impression of sparseness or compact massing which one receives, the variety or homogeneity of the constituents in color, size, form and the like, the arrangement or lack of order which they chance to show, as well as many other influences, probably unite in determining our judgment and in one proportion or another predispose us to an over- or under-estimation of the numbers involved.

One group of these factors has been analyzed and tested experimentally by Dr. Burnett.[1] The elements considered in Burnett's paper comprise what may in general be called the objective group of factors. To this series belong all those qualitative variations which mark the constitution of different groups of objects, such as brightness, color, size, form and distribution in space. There remains to be considered the nature and importance of another group of factors which may be called subjective or resident in character. These consist of the series of responses or modifications of attitude which the objective differences tend to provoke.

The existence of this resident factor need not be obvious to introspection. Though present as an effective determinant in the judgment it may form an indiscriminable element in a fused impression, as the complex of pressures and muscle-strains in

[1] Burnett: Harvard Psychological Studies, 1906, iv, 349.

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the mouth enters into our representation of the sourness of a taste without attracting attention to itself.

That such variations in the induced reaction exist in related fields of experience has been shown in regard to several forms of judgment. An interrupted line appears longer than an unbroken one lying within the same termini because the eye, in measuring the latter, makes a relatively continuous movement between the limits of its exploration, while in the former case each interruption tends to arrest or retard the eye, and thus imposes a greater quantity of effort in making the complete movement than is required in following the continuous line. So, too, in cases of partial paralysis of the rectus externus muscle the angle of actual rotation in the case of a given movement of the eye horizontally outward is over-estimated. With an incomplete radial swing the normal limit of reaction is supposed to have been reached on account of the excessive or maximum effort involved, and an illusion of direction results. The variations of the Muller-Lyer figure involve a like factor as Wundt and Delboeuf point out.

The general physiological conditions underlying this whole field of phenomena have been described by the writer in a previous paper.[2] Any visual field may be characterized as a dynamic system every point of which plays its part in determining the reaction of the eye.

Reflex movements are incessantly initiated by the appearance of strong local stimuli, and voluntary movements, with scarcely less frequency, are affected by the presence of unequal intensities and qualities of illumination in different parts of the field of view as the point of regard moves over it. Theoretically, it may be said that every bright point or object one sees either arouses a movement of the eye or inhibits it if it be in progress—including within the term any retardation of motion as well as its complete cessation. These unregarded factors, causing movement where none is intended or recognized and transforming it in greater or less degree where it is in progress, give rise to a variety of errors in our judgments of distance, direction, size, form and the like.[3]

[2] MacDougall: this Journal, 1903, ix, 122.
[3] Loc. cit.: p. 122.

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The paper in question considers only the influence of such factors upon our judgments of linear magnitudes through the variations which they induce in the system of eye-movements. Our judgments of size, form and direction arise in the same way in all cases where no exact determination is possible and we must depend upon the general impression which is received. Our "guessing" or approximative estimation of number is subject to the same general conditions. Such estimates are clearly affected by the objective factors already mentioned—dispersion over the field, grouping, color and other qualitative variation in the constituent objects, as Dr. Burnett has shown. The mode in which such objective differences work upon the mind so as to influence the judgment is a question that still remains to be considered, and this paper presents the results of an attempt to isolate an element of this second process—it may not be the sole one—and to show its influence in our judgments of number.

This factor is the tendency of the eye to respond involuntarily to the stimuli which the visual field presents—to come to rest or to move, to be accelerated or retarded in its motion—in ways which are determined by the whole character and distribution of objects in the visual field. If such influences affect our judgment arrangements of objects which predispose to a greater range or number of eye-movements should be over-estimated, while those which offer less stimulus to such reflex movements should be under-estimated in number.

This principle has been applied in a series of different conditions, comparison in each case being made between two groups of objects in one of which the arrangements—in relation to some specific characteristic—was such as to afford a greater stimulus to eye-movement than in the other. The series of comparisons included the factors of brilliancy of coloring, sharpness of contrast with the background, induced fixation and oscillation of the point of regard, confusion and order in arrangement, direction of eye-movements, etc.

The materials employed in the tests consisted of black, gray and orange squares of paper 1 cm. in diameter (Series I–VII;

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IX–X), and of strips of black paper 1.6 cm. in length by 0.2 cm. in width (Series VIII). In each test a fixed number of these scraps of paper, sufficiently large to make any process of counting impossible in the short period of observation allowed, was distributed over an area 9 cm. square. Side by side with this was arranged a second group scattered over the same area but differing from the first in some of the characteristics already referred to.

The number in this second group varying at each successive test the two arrangements were presented simultaneously upon a dull white background and the observer was required only to say, in relation to each comparison made, whether the number in the variable group appeared less than, equal to, or greater than that in the standard. The usual precautions against time and space errors were taken, and an interval of about three seconds was adopted as most suitable to securing a distinct impression of comparative number while obviating the possibility of counting the objects presented.

The series of tests was arranged as follows:

I. Comparison of numbers in areas filled with black squares: (a) unenclosed, and (b) enclosed by a band 2 mm. in width.

II. Comparison of numbers in areas: (a) having all squares alike and black, and (b) having a single orange square in the centre of a field of black squares.

III. Comparison of numbers in areas: (a) having black squares arranged in regular order, and (b) having black squares scattered without arrangement.

IV. Comparison of numbers in areas: (a) having black squares arranged to form a unitary figure, and (b) having black squares scattered without arrangement.

V. Comparison of numbers in areas: (a) having all squares alike and black, and (b) having all squares alike and gray.

VI. Comparison of numbers in areas: (a) having two vertical columns of squares in a field otherwise without order, and (b) having two horizontal rows of squares in a field otherwise without order.

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VII. Comparison of numbers in areas (a) arranged throughout in vertical columns, and (b) arranged throughout in horizontal rows.

VIII. Comparison of numbers in areas: (a) having strips of black paper with their axes vertical, and (b) having strips of black paper with their axes horizontal.

IX. Judgment in absolute terms of numbers in areas: (a) having an orange square in the center of a field of black squares, and (b) having an orange square at the middle of each side, right and left, of the field of black squares.

X. Judgment in absolute terms of numbers in areas: (a) when the centre of the field was fixated and wandering of the eye deliberately suppressed, and (b) when the eyes were systematically moved about the field during observation.

Three arrangements having a greater number of objects and three having a smaller, as well as one having the same number, were compared with the standard group. Each of these seven comparisons was made twice in a continuous series of experiments, the numbers in the variable group being presented in irregular order and their arrangement within the series changed after each group of fourteen judgments. Five such series of individual comparisons were made in each of eight of the ten sets of experiments. In two supplementary series (IX and X) with which the investigation closed eight different groups of objects were employed some of which were estimated twice, others three times, in a continuous series of judgments, the number of such series in the two arrangements being twelve. Three subjects were tested in Series I-II; four in Series III-VIII; and three in Series IX–X. The number of tests made is thus: in the first group, 420; in the second, 1680; and in the last, 924—a total of 3024. The number of individual comparisons made in each case is therefore sufficient to secure a typical reaction, especially in view of the conformity which appears in the judgments of the various observers who took part in the investigation.

For convenience in presenting the results a conventional method of securing a quantitative expression has been adopted, namely, by assigning to each of the three classes of judgments—

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less, equal, greater—a position in an ordinal series having the respective values of one, two and three. Thus, if the series of ten comparisons of a variable group of eighteen with the standard of twenty resulted in six judgments of "less," three of "equal," and one of "greater," the result would be expressed by the number 15, computed by multiplying the 6 by unity, the 3 by 2, and the 1 by 3, and adding their products. The quantities thus obtained were then compared with a standard series representing the distribution of errors to be expected within the thresholds of clear discrimination selected as limits to the range of number-variations used. Thus in the series having twenty objects in the standard group seventeen and twenty-three objects were selected as the limits of variation after tests which showed these numbers to be just beyond the inferior and superior discrimination—thresholds respectively for groups varying in a purely quantitative way.

The ideal series of values taken to represent these limits and the numbers lying between them were as follows: 10.0; 13.3; 16.6; 20.0; 23.3; 26.6; 30.0. The first of this series records a group of judgments representing the inferior limit in which each of ten reactions it contained had a value of 1—that is, in which all were *minus* judgments. The last, likewise, records a series representing the superior limit. The value of 20 assigned to the fourth place in this series (in which the variable group contains the same number of objects as the standard) can appear in the quantitative valuation of the results only (a) when every judgment in the series has been correct, or (b) when there is an equal distribution of errors on either side.

The results of the tests made on each individual are expressed in the tables in terms of the series of variations from this ideal curve which they present. The algebraic sums of the *plus* and *minus* errors thus obtained from each individual are then combined and the final result presented in the form of an average tendency to under- or over-estimation as measured by their (−) or (+) variation from the curve assumed as a standard

Throughout the following presentation of results it is to be understood that the variable is the arrangement assumed to have

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less value as a stimulus to eye-movement, and that the figures indicate whether this is under-estimated (minus-sign quantities) or over-estimated (plus-sign quantities). In Table I the results of the first series of tests are presented, the upper lines showing the distribution of judgments for the subjects individually and the lower giving the summation of (+) and (−) errors for each, together with the average variation for the group.

TABLE I

SUBJECT	17	18	19	20	21	22	23
M	± 0.0	− 3.3	− 3.6	+ 4.0	+ 0.7	+ 3.4	± 0.0
K	± 0.0	− 3.3	− 5.6	+ 1.0	+ 2.7	+ 0.4	± 0.0
R	± 0.0	− 2.3	− 4.6	− 3.0	− 1.3	+ 2.4	± 0.0
M − 6.8		K − 4.8		R − 8.8		Av. − 6.8	

It will be seen that to every subject the number of squares in these uncounted groups appears smaller when they were framed by a black margin than when left unenclosed. Now any such border has the effect of defining the limits of the group as an object of regard; a picture for example, is thus isolated from its surroundings as an element of technique. The introduction of such a positive visual boundary allows the eye to come to rest at what may be called the psychological centre of gravity of the whole group. It is by such means—balance of sensory factors such as mass, color, motion—that the painter contrives, through composition, to centre the eye upon the point of primary significance in his picture. In the unenclosed group on the other hand—if this be true—the eye will seek first in one direction then in another for the actual limits of the group with which it is called upon to deal. Thus in the very act of defining its object a greater number of explorative movements will be involved and the basis of a possible illusion laid.

In Table II the result of the second test is shown. As in the first series, all subjects show *minus* reactions; the group of black squares having an orange spot in the centre of the field is consistently under-estimated when compared with another group differing only in having all squares alike black.

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TABLE II

SUBJECT	17	18	19	20	21	22	23
M	± 0.0	− 3.3	− 6.6	− 9.0	5.3	+ 1.4	± 0.0
K	± 0.0	− 2.3	− 3.6	+ 3.0	1.7	+ 1.4	− 1.0
R	± 0.0	− 3.3	− 5.6	− 5.0	2.3	− 1.6	± 0.0
M − 22.8		K − 0.8		R − 17.8		Av. − 13.8	

Instead of depending upon the centering effect of an enclosing frame the immobilizing of the eye is here attained through the positive attraction of a vivid spot in the centre of an otherwise colorless and monotonous field of objects. One has only to recall the involuntary reaction of the eye in turning toward any point of illumination which suddenly appears in a dark field in order to realize the arresting effect of such a stimulation-point as the spot of orange in its surroundings of black. The greater efficiency of this variation is indicated by the difference in amount of average error, that of Series II being more than double the error of Series I.

In Table III are given the results of comparing a group of black squares in which all orderly arrangement was avoided with one in which the squares were disposed checker-board fashion in basal and diagonal lines, both groups being unenclosed.

TABLE III

SUBJECT	18	19	20	21	22	23	24
M	± 0.0	− 3.3	− 6.6	− 10.0	− 6.3	+ 3.4	± 0.0
K	± 0.0	− 1.3	− 4.6	− 1.0	− 0.3	+ 0.6	− 1.0
R	± 0.0	− 3.3	− 2.6	+ 1.0	− 2.3	− 2.4	− 1.0
T	± 0.0	− 2.3	− 1.6	− 2.0	− 3.3	− 0.6	± 0.0
M − 22.8		K − 6.8		R − 7.8		T − 9.8	Av. − 11.8

When any group of objects irregularly distributed over a field is presented to sight, the eye habitually moves in a desultory way from point to point as each individual attracts it in succession. In such cases we may notice how the eye is seized and led along by any well-defined alignment of materials or objects,

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such as a river or range of hills in the landscape, the cornice or a row of windows in a building, a series of footsteps in the snow and the like. It is the object of this series of tests to isolate this characteristic reaction of the eye and to test its presence as a factor in our judgments of number.

In the fourth series this principle is merely varied in form and repeated, the squares in one group being without arrangement as before, while in the other they form a linear pattern or figure. The results are shown in Table IV.

TABLE IV

SUBJECT	18	19	20	21	22	23	24
M	± 0.0	− 3.3	− 6.6	− 10.0	− 13.3	− 15.6	−16. 0
K	± 0.0	− 2.3	− 1.6	± 0.0	+ 0.7	+ 1.4	− 2.0
R	± 0.0	− 3.3	− 3.6	− 4.0	− 0.3	− 0.6	± 0.0
T	± 0.0	− 3.3	− 2.3	± 0.0	+ 2.7	+ 0.6	± 0.0

M − 64.8 K − 3.8 R − 11.8 T − 2.3 Av. − 20.7

In this case it seemed to the experimenter that the situation might involve at least two factors in potential opposition to each other, and that the question of predominance could not easily be determined in advance. The second element is the unitary pattern which the squares formed. If this were at once apprehended in its totality it might have the effect of inhibiting explorative movements instead of stimulating them. The figures, however, seem to show not only that the lines had their characteristic effect but that the increased definition of the line arrangement intensified the illusion, the index of error being almost twice as great as in the preceding test. (In these two series, on account of the requirements of arrangement, 21 squares were used in the standard instead of 20.)

In the remaining series of this part of the investigation the number of squares in the standard group was raised to 30. Though there had not been any counting of the smaller numbers, one or two of the reactors had spoken of the rise of a certain feeling of familiarity or control in regard to the groups after a fairly large number of judgments had been made upon them

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TABLE V

SUBJECT	24	26	28	30	32	34	36
M	± 0.0	− 3.3	− 6.6	− 8.0	− 5.3	− 3.6	− 3.0
K	± 0.0	− 3.3	− 6.6	− 8.0	− 7.3	− 5.6	− 6.0
R	± 0.0	− 3.3	− 6.6	− 5.0	− 4.3	+ 0.4	± 0.0
T	± 0.0	− 3.3	− 5.6	− 5.0	− 3.3	− 0.6	− 1.0

M − 29.8 K − 36.8 R − 12.8 T − 18.8 Av. − 24.3

and in order to preserve as fully as possible the guessing attitude this increase was considered desirable.

In the fifth series, the results of which appear above, the squares in each group were homogeneous and distributed without order, but in the one case they were black and in the other neutral gray. The difference was like that between flocks of crows and of buntings on a snow field, and the magnitude of the illusion was doubtlessly increased by the passing of the eye directly from one group to the other in making judgment. As compared with the sharp individuality of the squares of black against their white ground the gray had a suggestion of what may be called texture rather than multiplicity. The effect of this arrangement in thus reducing the stimulation value of the whole group as contrasted with increasing that of the rival group by a single vivid square (as in Series II), was to raise the error of under-estimation to the highest degree reached in the whole series of tests, an average of −24.3.

In the sixth series, shown below, one-quarter of the total number of squares in each of the two groups were arranged in two rows, three-quarters of the number being scattered irregu-

TABLE VI

SUBJECT	24	26	28	30	32	34	36
M	± 0.0	− 3.3	− 6.6	− 10.0	− 13.3	− 16.6	− 15.0
K	± 0.0	− 3.3	− 5.6	± 0.0	− 1.3	− 1.6	− 1.0
R	± 0.0	− 3.3	− 4.6	− 1.0	− 0.3	− 0.6	± 0.0
T	± 0.0	− 3.3	− 1.6	− 1.0	+ 3.6	+ 1.4	± 0.0

M − 64.8 K − 12.9 R − 9.9 T − 1.0 Av. − 22.1

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larly over the remaining field. In the standard group the rows thus made ran vertically while in the variable they were horizontal.

The assumption which this arrangement was designed to test is that the greater facility of right and left eye-movements as compared with those in a perpendicular direction should have the effect of producing an illusion of judgment (our estimates of numbers being influenced by explorative movements) if the amount of effort involved function as a measure of the quantity of such movement.

In Series VII an accentuation of this principle of arrangement is applied. All the squares were there arranged in line. In the standard group these were vertical, care being taken to avoid any regular horizontal or diagonal pattern, while in the variable the arrangement showed a sweep of horizontal lines alone. The figures are given in Table VII.

TABLE VII

SUBJECT	24	26	28	30	32	34	36
M	± 0.0	− 3.3	− 6.6	− 4.0	− 8.3	− 2.6	− 2.0
K	± 0.0	− 2.3	+ 0.4	+ 2.0	+ 1.7	+ 1.4	± 0.0
R	± 0.0	− 2.3	− 4.6	+ 4.0	+ 2.7	+ 0.4	± 0.0
T	± 0.0	− 3.3	+ 0.4	− 2.0	+ 0.7	+ 1.4	± 0.0
M − 26.8	K + 3.2	R + 0.2	T + 0.4	Av. − 5.7			

It will be noted that this emphasis of line arrangement has not, in this case, resulted in an increase of the illusion as in Series IV. The complementary factor there mentioned—apprehension of a unitary pattern in the arrangement—may possibly be the cause of this.

In the last series of this group—Table VIII— the same principle was modified in a new direction. For the squares were substituted minute strips of black paper, about one-quarter of the diameter of the field in length. These were strung in rows which ran vertically in the one arrangement and horizontally in the other but did not with their edges form an exactly aligned square. The prevalence of under-estimation in the latter case

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TABLE VIII

SUBJECT	24	26	28	30	32	34	36
M	± 0.0	− 3.3	− 6.6	− 6.0	− 7.3	− 5.6	− 2.0
K	± 0.0	− 3.3	− 6.6	− 7.0	− 1.3	− 5.6	− 1.0
R	± 0.0	− 3.3	− 2.6	+ 4.0	+ 6.7	+ 3.4	± 0.0
T	± 0.0	− 3.3	+ 0.4	− 3.0	− 1.3	+ 3.4	± 0.0
M − 30.8	K − 24.8	R + 8.2	T −3.8	Av. − 12.8			

is obvious, though one of the four subjects (R) presents a positive error of considerable magnitude.

In all three series of this group the arrangement of the variable field is designed to stimulate horizontal movements of the eye and that of the standard to stimulate its elevation and depression. The normal field of exploration in vision may be described as a quadrant lying between the horizon and the feet. Within this area are to be found most of those things to which it is practically necessary to attend because they are supported by the general surface of the earth. Over this area the eye incessantly sweeps, including in its right and left rotation a field of more than 180° exclusive of head movements, while its vertical rotation in passing from a point near the feet to one on the extreme horizon is less than 90°.

Above the level of the eyes few objects are to be found which habitually attract our attention. To the region of the sky we look for weather signs and occasionally the beauty of the cloud or of a bird's flight draws the gaze. The mere movement of elevation is difficult and its increasing strain as the eyes are raised soon leads to a backward rotation of the head. The sense of sublimity in mountains must be attributed in part to the effort of looking up to them and the fatigue it involves. That the eye tends to seek rest in a point of regard below its own level is indicated by the depression of the subjective horizon which was found in an earlier investigation of the writer's.[4] In this greater facility of horizontal movements is to be found, at least in part, an explanation of the illusion of proportion in a geo-

[4] MacDougall: Harvard Psychological Studies, 1902, i, 145.

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metrical square, with which we are also familiar in the greater apparent length of a post when set on end than lying flat.

In general we may conclude that when equal quantities of eye movement are induced in two cases, one of which is predominantly horizontal and the other vertical, the latter will give rise to a greater sense of effort and afford a basis for just such an illusion as these three series of judgments present, if such explorative reactions do constitute a factor in our estimates of number.

The investigation closed with two series in which the form of judgment was changed to allow an estimation in terms of

TABLE IX

SUBJECT	K		R		T	
Arrangement	A	B	A	B	A	B
20-group	197	202	201	205	201	203
	197	197	198	200	196	199
Totals	394	399	399	405	397	402
30-group	356	356	333	344	369	350
	341	349	346	370	347	356
	345	364	341	354	350	368
Totals	1042	1069	1020	1068	1066	1074

absolute numbers. The first of these also introduced a new variation in the stimulus to movement. In the ninth test two groups of irregularly spaced black squares were arranged, one of which (Table IX, A) had an orange square in the centre of the field (as in Series II), while the other (Table IX, B) had two such squares, one at each side of the square half-way up.

At first, in both this and the final series, each group was composed of 20 squares, but later the number was raised to 30 as in Series V–VIII. The subject being asked to guess the actual number of squares seen in these two series there was of course no comparison of standard and variable; but further, the number of objects in the group was not changed from exposure to expos-

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ure, novelty being secured by rotating the field through 90° after each judgment.

The results—Table IX—are expressed in terms of the sum of the estimates made in the course of a single series of judgments. The number of judgments thus combined is, in the 20-group, ten; and in the 30-group, twelve. The sum of all estimates made is set down in the totals for each group. The figures show that in each arrangement every observer judged the group which had an orange square at each side of the field, right and left, to be numerically greater than that which had but one central

TABLE X

SUBJECT	K		R		T	
Arrangement	A	B	A	B	A	B
20-group	215	209	214	241	197	199
	199	202	204	212	199	199
	200	200	199	204	200	200
	200	199	204	202	202	197
	201	199	199	202	200	200.
Totals	1015	1009	1020	1061	998	995
30-group	416	393	295	328	366	350
	412	386	315	327	305	294
	391	388	302	305	302	302
	380	376	311	301	306	302
Totals	1599	1543	1223	1261	1279	1248

point of such vivid stimulation. The difference is not large but it is highly constant, only one individual series of judgments out of fifteen reversing this relation.

In the final series of tests a new element of the problem was attacked, and though the data secured—as shown in Table X—afforded an insufficient basis for general conclusions the results verify the assumption they were designed to test.

In the preceding series the observer was simply asked to estimate the number of squares seen at each exposure, the question of method in obtaining his results being purposely kept in abey-

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ance, as the real nature of the problem under investigation had been concealed from the beginning. In the tenth series on the contrary, the subject was asked specifically to modify his observation of the field. Under arrangement (A) he was directed to fixate a point in the centre of the area and attempt to get a general impression of the number of squares without exploring the field. Under arrangement (B) the observer was directed to look carefully over the field, moving the eye back and forth throughout its extent so as to get a more detailed knowledge of the size and distribution of the group of objects.

In other words the test was designed to isolate the effect of introducing 'forced'—i.e., deliberate—eye-movements into the observation of the field, as contrasted with those unreflective wanderings which ordinarily occur as the eye is attracted to point after point in its habitual exploration of such a field. A comparison of the (A) and (B) judgments shows no such constant error as that which appears in Table IX and is found elsewhere throughout this investigation. Reversions mark the estimate of every observer, in one series (A) will be judged greater, in another (B). Further the table shows 14 series in which (A) is set higher, to 8 in which (B) appears greater. The first total sustains this preponderance (A, 7134; B, 7117); under condition (A) the number of squares—to all observers together—appears greater than under (B). The effect of introducing these deliberate eye-movements, practically negligible though it be, is actually to reduce the estimate. In passing, it is worthy of note that under the systematic exploration which (B) calls for the resultant estimate is on the whole more accurate than when the eye is fixed, though the difference is very slight.

SUMMARY

These experiments seem, on the whole, sufficient to justify the belief that explorative movements of the eye in reviewing any group of objects do enter into our common judgments of the number composing it. The factors of any such judgment therefore fall into two groups as suggested, the transitive and the

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resident. The former includes all such objective variations as differences in color, brightness and size; the latter consists in the complex of kinaesthetic sensations aroused in connection with the perception. On the relative importance of these two groups of elements in any such judgment the present investigation does not throw light. In normal experience they are habitually correlated; the eye-movements are stimulated by differences in the material.

But it is to be noted that such differences are not to be restricted to the elements of color and brightness, shape and size of the individual objects. They include also distribution, grouping, pattern, the general drift and relation of suggested lines, in short what may be called the element of form. The individuals of any group having a given qualitative variety are susceptible to rearrangements which will either increase or diminish their apparent number, this modification being dependent upon the stimulative effect of the system in arousing eye-movements or their traces.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXIII. The Cause of the Variations in the Gastric Hunger Contractions with Age

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INTRODUCTORY

In an earlier paper (1) it has been shown by starving young and old animals that in very young dogs the hunger contractions of the empty stomach are practically continuous, while in old animals there is a decrease in the gastric hunger activity proportional to the advance in age. This decrease is shown to some extent in the tonus, in the strength and rapidity of the hunger contractions, and especially in the duration of the hunger and quiescence periods of the stomach. The present investigation deals with the cause of these variations, as well as with the effects produced upon them during and in prolonged starvation. The previous work had already seemed to indicate that the activity of the gastric motor mechanism was dependent not only upon the actual age but upon a correlation which exists between the gastric hunger mechanism and rate of metabolism of the animals at different ages, which with the above mentioned variations in connection with the present work makes it possible to differentiate between these two factors.

Oxidation of matter is always going on in the living cells of the gastric mechanism, as well as in the other living cells throughout the body, and the energy liberated in these decompositions must be the power upon which the hunger movements depend.

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In this process of endogenous gastric metabolism there is a constant state of partial breaking down and materials must be furnished to repair the worn-out parts, and so in the fuel factor and the repair factor may lie the metabolic correlation of the gastric hunger activity, which in connection with the age factor of retarded activity may give rise to the gastric hunger variations with age. A gradual decline in the strength of the hunger contractions after the first few days of starvation with an increase in the gastric tonus was expected, but the contractions in the extreme stages of starvation instead of reaching the zero limit and remaining there until the death of the animal surprisedly showed periodic outbreaks with the return of the strong hunger contractions. In other words, it is probable that a short time before the complete break-down and death of the animal organism, due either to the body's own store of fuel becoming exhausted, or to a part of the bodily machinery necessary for life wearing out the gastric hunger mechanism itself may become an automatic centre independent of the central nervous system.

EXPERIMENTAL PROCEDURE

The dogs used in these experiments were provided with the simple gastric fistula (2), and the records of the gastric hunger contractions were taken by the ordinary balloon method with the animals lying quietly in the lap. Three groups of dogs were selected, each group being composed of an old adult and a young pup five to six months of age. The starvation periods were continued for seven days (one week), and daily records commencing after the first twenty-four hours of starvation were taken on the old and young dogs, respectively. At the end of the week's fast they were given one week to recover and were fed on light food (bread and milk) for the first time or two and in relatively small amounts. Controls were run on the first two groups of dogs, but not on the third group, for the reason, that it did not appear necessary, since the results were so uniform and conclusive. In the case of the first group it was possible to obtain dogs of the same species (fox terriers) and sex (females),

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but in the latter two groups the species and sex differed; the old dogs in both cases being females and the young pups males.

In addition to the above, the nature of the gastric hunger contractions were studied in prolonged starvation. For this part of the investigation two of the young pups in the above mentioned series (first and third groups) were selected and the starvation continued until the death of the animals, which in the first case, took place on the fifteenth, and in the latter case, on the thirteenth day of starvation. However, in the latter case, both the old and young dogs of the third group were treated likewise, principally to show the greater endurance of the older animal. In the extreme stages of starvation, or three or four days before death the strictest care of the animals had to be observed, since they became so weak that they were unable to attend to the welfare of their bodies, and so in order to guard against infections and to keep the animals in a perfectly healthy condition it became necessary to wash out the eyes, nares, and other body openings several times daily and help the animals in numerous other ways. The gastric tonus was determined by strongly inflating the balloon after its introduction into the stomach and then letting the pressure fall to approximately 3 cm., this being the constant pressure used throughout all the experimentation.

RESULTS

Prolonged Type III Hunger Contractions

The hunger contractions of the empty stomach as registered by the delicate balloon method have been classified by Carlson (3) into three distinct types, namely, Types I, II, and III, each of which are dependent upon a particular degree of stomach tonus. However, in the extreme stages of starvation there may be a prolonged Type III hunger contraction which also must be dependent upon a certain degree of stomach tonus. This exaggerated type of hunger contractions appears to be of very short duration and is present only during the last 48 to 60 hours previous to the death of the animal from prolonged starvation as indicated by my records. It always occurs in Type III hunger

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contractions (incomplete tetanus or strong stomach tonus) and consists of a very abrupt outbreak represented by an enormous increase in gastric tonus of the composition of incomplete tetanus which may even approach complete tetanus, superimposed upon the Type III contractions, which is then followed for a brief period after the relaxation from the extra tonus by hunger contractions characteristic of Type II on the tonus of Type III contractions which gradually changes back into the true Type III contractions without any appreciable change in the gastric tonus. The length of the tetany periods of these

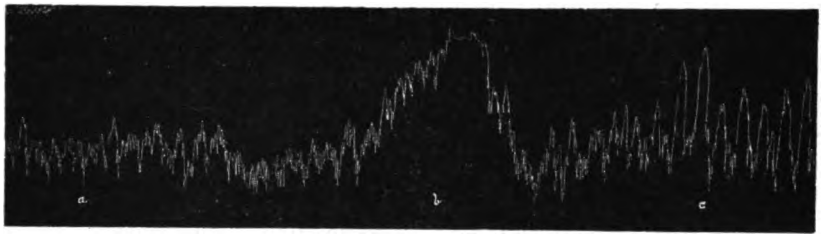


Fig. 1. About two-fifths the natural size. Tracing showing tonus and hunger contractions of young Dog B (Series I—control) after 360 hours (15 days) starvation; a, Type III contractions; b and c, prolonged Type III contractions showing strong tetany portion followed by characteristic Type II contractions on the tonus of Type III. Chloroform manometer. Black line below tracing = 0 mm. pressure.

prolonged Type III hunger contractions as shown from the drum records varies from about two and a half to four minutes, and the duration of the entire period including the tetanus and the characteristic Type II contractions varies from about five to thirteen minutes. These periods gradually increase in length from their first appearance with the increase of the starvation until just a very few hours before death when the gastric motor mechanism seems to loose its activity. A typical tracing illustrating this type of hunger contractions is reproduced in figure 1. In the following brief protocols and elsewhere this exaggerated

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type of hunger contractions will be referred to as the prolonged Type III hunger contractions, the probable cause of which will be discussed more in detail later.

SERIES I. DOGS A AND B. CONTROL EXPERIMENTS

Dog A (very old adult):

July 20. Fast 29 hours. Type II hunger contractions (feeble). Gastric tonus 3 cm. with increase to 4 cm. Slight tonus rhythm.

July 21. Fast 52 hours. Type II contractions. Gastric tonus 3 cm. with increase to 4¼ cm. Slight tonus rhythm.

July 22. Fast 76 hours. Type II contractions. Gastric tonus 3 cm. with increase to 4¼ cm. Slight tonus rhythm.

July 23. Fast 96 hours. Type III contractions. Gastric tonus 3 cm. with increase to 4½ cm. Very slight tonus rhythm.

July 24. Fast 125 hours. Type III contractions (feeble). Gastric tonus 3 cm. with increase to 3½ cm. Slight tonus rhythm. (Dog rather depressed, sore eyes, etc.).

July 25. Fast 148 hours. Complete absence of hunger contractions. Gastric tonus remains constant at 3 cm. No gastric tonus rhythm. (Dog ill with general dog distemper—heavy respiration).

July 26. Dog found dead in the morning.

Dog B (young pup 5–6 months):

July 20. Fast 30 hours. Type II contractions. Gastric tonus 3 cm. with increase to 4 cm. Slight tonus rhythm.

July 21. Fast 53 hours. Type II contractions. Gastric tonus 3 cm. with increase to 4½ cm. Slight tonus rhythm.

July 22. Fast 77 hours. Type II contractions (vigorous). Gastric tonus 3 cm. with increase to 4¾ cm. Slight tonus rhythm.

July 23. Fast 97 hours. Type II contractions. Gastric tonus 3 cm. with increase to 4½ cm. Slight tonus rhythm.

July 24. Fast 131 hours. Type II and III contractions. Gastric tonus 3 cm. with increase to 4¾ cm. Slight tonus rhythm.

July 25. Fast 150 hours. Type II and III contractions. Gastric tonus 3 cm. with increase to 4½ cm. Very slight tonus rhythm.

July 26. Fast 169 hours. Type II and III contractions. Gastric tonus 3 cm. with increase to 5¼ cm. Very slight tonus rhythm.

July 27. Fast 191 hours. Type II and III contractions. Gastric tonus 3 cm. with increase to 6¼ cm. Very slight tonus rhythm.

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July 28. Fast 215 hours. Type III contractions. Gastric tonus 3 cm. with increase to 7 cm. Slight tonus rhythm.

July 29. Fast 239 hours. Type III contractions. Gastric tonus 3 cm. with increase to 6½ cm. Very slight tonus rhythm.

July 30. Fast 263 hours. Type III contractions. Gastric tonus 3 cm. with increase to 6½ cm. Slight tonus rhythm.

July 31. Fast 287 hours. Type III contractions. Gastric tonus 3 cm. with increase to 6¼ cm. Slight tonus rhythm.

August 1. Fast 317 hours. Type III and prolonged Type III contractions. Gastric tonus 3 cm. with increase to 6 cm. in Type III and to 7 cm. in tetany portion of prolonged Type III contractions, the characteristic Type II contractions keeping the same gastric tonus as Type III. Length of complete period prolonged Type III contractions 6 minutes; length of tetany portion 2½ minutes. Slight tonus rhythm.

August 2. Fast 336 hours. Type III contractions. Gastric tonus 3 cm. with increase to 6½ cm. Very slight tonus rhythm.

August 3. Fast 360 hours (15 days). Type III and prolonged Type III contractions. Gastric tonus 3 cm. with increase to 5 cm. in Type III and to 9¼ cm. in tetany portion of prolonged Type III contractions, the characteristic Type II contractions keeping the same gastric tonus as Type III. Length of complete period prolonged Type III contractions 13 minutes; length of tetany portion 4 minutes. Very slight tonus rhythm. (See fig. 1).

August 3. Fast 368 hours (15⅓ days). Complete absence of hunger contractions. Gastric tonus remains constant at 3 cm. No gastric tonus rhythm, but a marked respiration. (At the time this record was taken the pup was in such a weakened condition from the long fast that the tracing was concluded after 30 minutes when the animal passed into vomiting spasms.) The pup died in about one hour without the slightest struggle, apparent pain, or discomfort—simply worn-out.

The protocols for Series II (Dogs C and D), Series I (Dogs A and B—first experiments), and Series III (Dogs E and F) are not given, since the same general characteristics are presented as in the preceding tabulations.

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DISCUSSION OF THE VARIATIONS IN THE GASTRIC HUNGER CONTRACTIONS

The condition of the stomach's activity if dependent alone upon the actual age of the gastric motor mechanism ought to increase in old animals with the starvation until it equalled that in the young animals, but since this is not the case, as is shown throughout the series of experiments we must interpret the variations in the gastric hunger contractions as being produced not only through the factor of age but also through the rate of metabolism. This latter factor appears to be of the greater importance in determining the activity of the gastric motor mechanism. Therefore, this retarded activity as seen in the gastric hunger contractions of old animals must be due to a slower rate of metabolism brought about and controlled chiefly through the age factor. There was a slight increase in the amplitudes of the hunger contractions during the first few days of starvation followed by a gradual decline. This slight increase occurred in all the dogs with but one exception, namely, in the old dog of Series I, first experiments in which the hunger contractions were feeble. This particular animal was very old and the party from whom she was obtained claimed that she was over eleven years of age and this statement was probably authentic, since her general appearance and loss of teeth indicated it. The old dogs of the other two series were younger (probably not over seven or eight years of age), and the hunger contractions were vigorous and did not show this feeble type.

The feeble contractions in this very old dog are probably identical with the contractions in those animals with which Boldireff (4) worked for like his, the hunger contractions were feeble during the first few days (first experiment, Series I—Dog A), then there was a slight increase in the amplitude followed by the usual and gradual decline. This increase in the strength of the hunger contractions after the first few days of starvation does not corroborate entirely with the results of Boldireff, since he states that after the first three or four days there is a copious and continuous secretion of gastric juice, which, of course,

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will lead to the inhibition of the hunger contractions if the normal percentage of HCl is present (5). The small and feeble contractions during the first few days may possibly be explained by supposing that there is a hypersecretion of the gastric juice sufficient to produce a partial inhibition of the hunger contractions for there seems to be no question but what there is a secretion of gastric juice in this animal during the first few days at least, judging from the quantity of fluid that escaped through the fistula, and besides the animal appeared perfectly normal. Later there is the increase in the strength of the contractions which may also be explained by assuming that the amount of NaCl in the blood, the principle source of the HCl of the gastric juice is greatly diminished by the complete elimination of the animal's food, the only source left for the NaCl being the very small quantity found in the water which the animals always had free access to. Thus, if the percentage of HCl is reduced in the gastric juice after the first few days the partial inhibition produced by it is also practically removed and we would expect the above increase in the strength of the hunger contractions which of only a brief duration is then followed by the characteristic and gradual decline.

A comparative study was made of the amplitudes of the hunger contractions in the old and young animals. In both the old and young dogs there appears to be an increase in the amplitudes of the hunger contractions during the first few days of starvation which is then followed by a gradual decline that continues to the death of the animal with the exception of a portion of the prolonged Type III contractions which appear only in the extreme stages of starvation. At no time do the amplitudes of the hunger contractions for the old dogs equal that of the younger ones, except in one case (Series III—Dogs E and F), where both the old and younger dogs were continued through a period of prolonged starvation until one of the animals died, and here on the ninth day of starvation the amplitudes of the old dog's contractions surpassed that of the pup, thus showing only the greater endurance of the older animal. In Series III—Dog E on the third day of starvation there was a decided fall in

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the amplitude of the old dog's contractions, but this was probably due to some disturbing influence, or perhaps to a slight depression in the animal, since it rose on the fourth and was continued on the fifth day to a degree which corresponded well with the second day's record. In Series I—control—Dog A too much stress should not be laid on the old dog's contractions, since she became ill on the fifth day with nose and eye infections which developed into a general pulmonary infection and died two days later. However, this did not seem to change materially the original results as shown by the first experiments on this animal, except that the amplitudes of the hunger contractions dropped more rapidly and somewhat lower after the animal

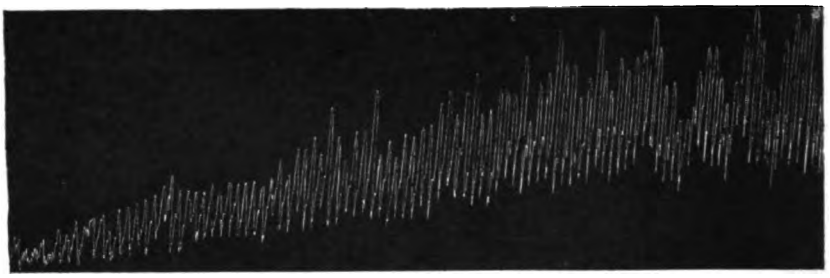


Fig. 2. About two-fifths the natural size. Tracing showing the hunger contractions of old Dog E (Series III) after 144 hours (6 days) starvation with a marked increase in the gastric tonus. Chloroform manometer. Black line below tracing = 0 mm. pressure.

became ill. The tonus was also as markedly affected as is shown by the protocols. This is an excellent example of the inhibition of the hunger contractions through depression.

The protocols show that there is a marked increase in the gastric tonus during starvation up to within a few hours of the death of the animal and this increase in the gastric tonus appears to be directly proportional to the decrease in the amplitudes of the hunger contractions (Fig. 3). It appears also that some

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dogs may exhibit a strong stomach tonus at the beginning as in Series III—Dog E, while others may show relatively low tonus as in Series I—Dog B, and these are subject to only slight variations as shown by the controls, but in all cases, the tendency is a gradual increase in the gastric tonus irrespective as to whether

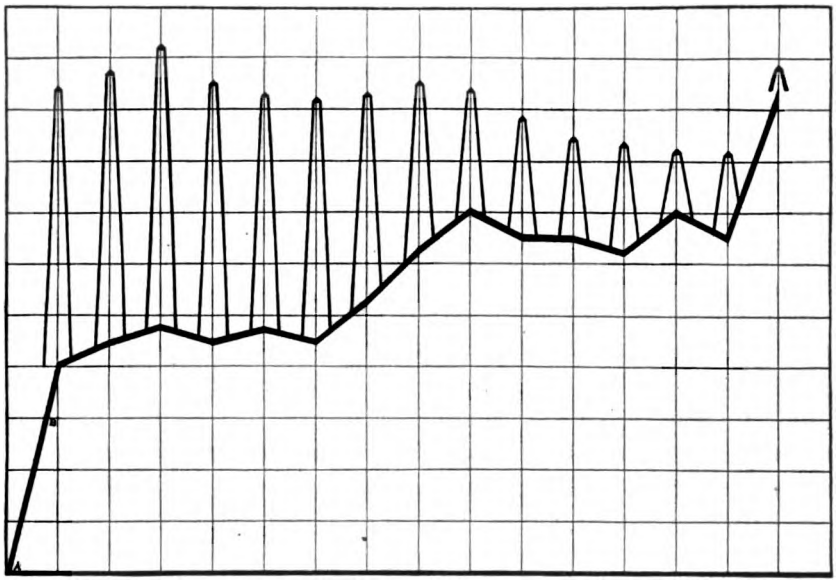


Fig. 3. Diagrammatic representation of the lowered amplitude of contraction on the base of the rising tonus as constructed from the daily tracings of young Dog B (Series I—control). Each of the above squares represent one sq. cm. The erect pyramids indicate the amplitudes of the hunger contractions in centimeters arranged on the rising tonus as a base line. Spaces left to right indicate number of days of starvation; spaces bottom to top daily increase in the gastric tonus in centimeters. Heavy line at bottom of chart = 0 mm. pressure of chloroform manometer. A to B = constant pressure of 3 cm. used throughout the experimentation. Note the rapid increase in gastric tonus and decline in amplitude of contractions on the fifteenth day as produced by the prolonged Type III hunger contractions.

the animal starts off on a high or a low gastric tonus. As has been stated before the different types of hunger contractions are dependent upon a particular degree of stomach tonus, and this increase in the tonus during starvation is accompanied by a progressiveness in the type of hunger contractions, that is, by

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an advance from the lower to the higher types respectively. To illustrate: If an animal's stomach starts off with Type II hunger contractions the tendency is a progression first to Type III contractions and then to prolonged Type III contractions. If it starts off with Type I contractions the progression is through the different types of hunger contractions from I to III inclusive. However, some dogs (Series III—Dog F) may show during the first few days of starvation an alternation between the Type I and II contractions, but the final result is always the same, namely, a progression from the lower to the higher types of hunger contractions. Therefore, the increase in the gastric tonus appears also to be directly proportional to the advance from the lower to the higher types of hunger contractions.

Prolonged starvation leads to increased activity of the gastric motor mechanism as indicated by the rise in tonus, at least, until that point is reached where the stomach becomes involved in the general debility and cachexia. Since the animals were allowed only water the energy necessary for their continued existence must have been met by the destruction of the stored up materials present in their own bodies as circulating and tissue protein, fat, and glycogen. The latter substance is quickly used up since it is present only in comparatively small quantities, and so the animal becomes emaciated since it lives upon its own body protein and fat. We know that the stored up body fats by a reversible lipolytic reaction may be converted over into soluble fats and oxidized by the cells to give heat and energy to the body, so also there is evidence of "circulating protein" in starvation as food for the tissues. Thus Miescher (6) showed that the salmon virtually starves, after entering the Rhine from the sea, whereas the genital organs of both the male and female greatly develop. This he claims is at the expense of the muscle tissue which to use the author's exact figures may loose as much as 54.74 per cent of their weight. Voit in his work on starving animals also believes in a "circulating protein," and, that the "organized protein" of the tissues themselves is gradually changed over in some way to the "circulating protein" and transported by the blood to the tissues needing nourishment. Luckhardt

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and Carlson (7) have reported on the possibility of a "hunger hormone" which gradually accumulates from the body tissues in general probably before the tissues actually demand additional fuel (food) which is transported by the blood to the gastric motor mechanism and there stimulates that organ to activity—hunger contractions. A gastric tonus rhythm is also evident which seems to be present in nearly the whole series of experiments (fig. 4). This tonus rhythm is probably produced by a reflex excitation through the motor nerve fibres which plays upon the oxidative processes in the tissues, thus augmenting at one time and inhibiting at another the physiological oxidations.



Fig. 4. About two-fifths the natural size. Tracing showing the hunger contractions of old Dog A (Series I) after 121 hours (5 days) starvation with a marked gastric tonus rhythm. Chloroform manometer. Black line below tracing = 0 mm. pressure.

LOSS OF HUNGER SENSATION WITH PERSISTENCE OF HUNGER CONTRACTIONS

Many cases of men and women have been cited in prolonged starvation by Carrington (8) where the hunger sensation subsided or disappeared after the third day. Viterbi, who voluntarily starved himself to death, noted complete absence of hunger after the fifth day (9), and the depression of this hunger sensation seems to be confirmed by the work of Carlson (10) on men and dogs. In dogs under observation it was noted that after the first four or five days of starvation there was a depression of the hunger sensation although the gastric hunger contractions persisted. This is explained by supposing that the neurones in the central nervous system which have to do with the

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sensation of hunger become fatigued after the first few days, thus eliminating entirely the hunger sensation, although the gastric hunger contractions are evident. In addition, to this loss of the hunger sensation the coming back or return of the strong hunger contractions as the prolonged Type III contractions must be considered, and the following considerations might be offered not only as a possible but also as a probable explanation. These characteristic hunger contractions which appear only in the extreme stages of starvation after the gastric motor mechanism has become hypersensitive from the long continued starvation must be due, either to a heat CO_2 stimulation from the protein of the circulation, or to an anemic stimulation of the stomach owing to the moribund state of the circulation, since the gastro-neuro-muscular apparatus has become entirely independent of the central nervous system. A typical tracing showing this supposed stimulating action of asphyxia or anemia on the hunger mechanism is reproduced in figure 1. Rogers (11) in his work on rabbits has spoken of prolonged contractions or periods of tetany lasting from two to three minutes in the last stages of starvation. These are probably identical with the prolonged Type III hunger contractions in dogs.

SUMMARY

1. The variations in the gastric hunger contractions are dependent upon two factors, namely: the actual age of the stomach and the rate of metabolism of the animal. The latter seems to be of the greater importance in determining the activity of the gastric motor mechanism.

2. The increase in gastric tonus during prolonged starvation is directly proportional to the advance from Type I to Type III hunger contractions, and inversely proportional to the decrease in the amplitudes of the hunger contractions.

The author wishes to express his thanks for the kind advice and criticism given him by Professor A. J. Carlson and for the library privileges offered him by the Johns Hopkins University.

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DIURNAL VARIATIONS IN ARTERIAL BLOOD PRESSURE

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From the Physiological Laboratory of Boston University

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INTRODUCTION

In the clinic, in the laboratory, in fact, wherever a physical examination is necessary, the determination of the arterial blood pressure is often important, so that a knowledge of the normal variations in pressure during the day is desirable in order that the determinations may be of practical value. Many of the investigations in this field have been incomplete, since the maximum pressure alone has been studied, and in all cases the number of subjects has been very small.

The literature on diurnal blood pressure seems to show that investigators have arrived at the following general conclusions: 1. There is a slight increase in the maximum blood pressure during the day. 2. Moderate muscular and psychical activities cause a more or less temporary rise in the maximum pressure. 3. The ingestion and digestion of food cause a rise in the maximum pressure, which remains at its height for a time, and then gradually falls until the next meal. 4. Maximum and minimum pressures are probably unequally affected by diurnal factors. 5. The pulse rate and the relative velocity of the blood flow (the product of the pulse pressure and the pulse rate, PP $\times$ PR, Erlanger and Hooker, 1904) seem to follow the variations in maximum pressure.

The purpose of this investigation was to make a study of the normal variations of the maximum, minimum and pulse pressures, the pulse rate, and the relative velocity of the blood flow during the day. In this paper we present the results of a series

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of observations on diurnal blood pressure in a group of healthy young men, all of about the same age and all living under identical conditions during the period of observation. Incidentally, the effect of ingestion and digestion of meals was demonstrated.

Because of the disconnected nature of the literature on this subject, an extended consideration of the work of other investigators may be taken up more advantageously after a description of our research.

SUBJECTS, APPARATUS AND METHOD OF PROCEDURE

Ten healthy male students were taken as subjects, who were all between 19 and 25 years of age.

An anaeroid sphygmomanometer was used, and the phases distinguished with a sphygmometroscope. The maximum pressure was read at the onset of the first phase, and the minimum was recorded at the beginning of the fourth phase, as the experiments of Warfield, 1913, and of the authors of this paper, 1913, seem to demonstrate conclusively that this is the point for reading the minimum pressure. All determinations were made on the right arm of the subject, who was so seated that the cuff of the sphygmomanometer was approximately on the level of the heart. The final readings that were recorded for each determination were the averages of three trials for each pressure. The pulse rate was determined at the wrist.

In order to accustom the subjects to the method of procedure and to insure normal readings by eliminating possible psychical effects, a preliminary period of observation on each subject was begun at 4.30 p.m. of the first day. Determinations were made at 5 p.m., 6 p.m., which was just before dinner, 6.45 p.m., which was just after the meal, 7. p.m., and every half hour until 9.30 p.m., which was just before the subject retired. The main period of observation continued throughout the whole of the following day. Determinations were made at 7.30 a.m., while the subject was seated in bed, 8 a.m., which was just before breakfast, 8.30 a.m., which was immediately after the meal, 9.a.m., which was after a ten-minute walk to the college, and every

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hour between classes until noon, when a determination was made just before luncheon. Observations were made at 12.30 p.m. immediately after the meal, 1 p.m., and hourly until 6 p.m., when determinations were made at the same intervals as on the previous evening until 9 p.m.

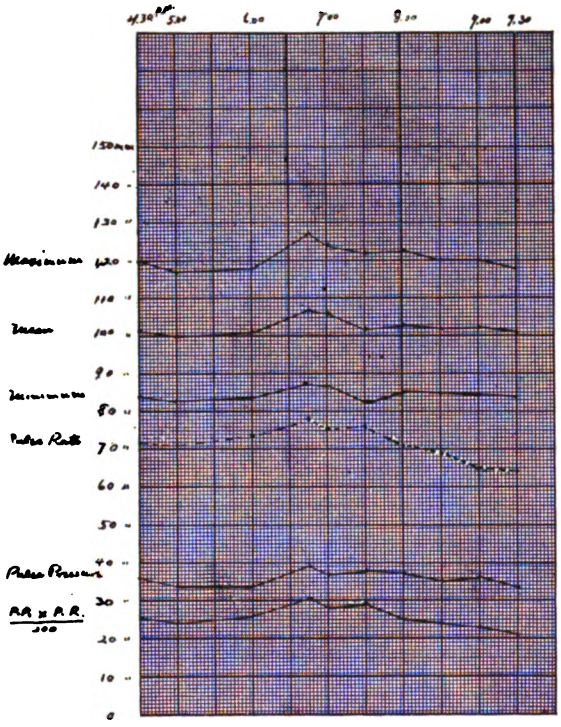


Plate 1. Average curves of the ten subjects for the preliminary period of observation. The readings at 6.00 p.m. were just before dinner, and at 6.45 just after the meal.

The students did the usual amount of study and class room work during the period of observation, but refrained from any unusual muscular exercise, for it has been shown by McCurdy (1901), Lowsley (1911), and Otis (1912), etc., that exercise causes a rise in the maximum pressure. Erlanger and Hooker (1904) state that both severe and moderate exercise cause an increase in the pulse pressure, pulse rate, and relative velocity of the blood

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flow, although moderate muscular exertion diminishes the minimum pressure, and severe muscular exercise increases it.

The morning meal consisted of fruit, cereal, toast, and coffee. Luncheon was a little larger, consisting of a stew or chowder, with bread, cocoa or milk, and dessert. The evening meal was the largest of all and consisted of a dinner of several courses. All the subjects ate their meals at the same place during the observations in order to make the conditions as nearly uniform as possible, and detailed records of the mental and physical activities of the subjects were kept.

RESULTS

The general average maximum blood pressure obtained by taking the average of all the readings throughout the periods of observation on all the subjects is 120 mm. of Hg.; the general average minimum is 85 mm. of Hg. This gives an average pulse pressure of 35 mm. of Hg. The average pulse rate is 72 beats per minute.

It will be seen that the average maximum and minimum pressures obtained in this investigation are slightly higher than those averages reported by some investigators. Thus Howell, in the fifth edition of his Text-Book of Physiology, states: "with his more complete apparatus Erlanger reports that in the adult (20 to 25 years) when the psychical factor is excluded, the average pressure in the brachial is 110 mm., systolic, and 65 mm., diastolic—figures much lower than those given by Potain. Von Recklinghausen's figures for the same artery are, systolic pressure 116 mm. of Hg., diastolic pressure 73 mm. of Hg." At no time during the day does our average curve fall as low as 110 mm., the lowest point being 114 mm. The significance of an average blood pressure is questionable, but it will be noted that in these observations the average pulse rate is 72, which is generally accepted as the normal for an adult man. Hence it would be fair to attach the same importance to a maximum pressure of 120 mm. as to a pulse rate of 72, recognizing that there may be a considerable variation, either above or below, that must still be

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regarded as normal for the individual. In these observations the averages obtained will tend to approach the true general averages since the former incorporate all the changes during the twenty-four hours except those during sleep. Such averages might possibly differ from those in which the readings were taken at a given time on successive days. This may possibly account for some of the differences in average blood pressure recorded by various observers. These differences are considerable. Thus, Janeway (1904) gives the maximum pressure as 100 to 130 mm. of Hg for individuals before middle life; Woley (1910) records a pressure of 122 mm. for individuals from 15 to 30 years of age, and 127.5 as an average for subjects of all ages; Cowing (1913) reports maximum pressures of from 85 to 110 mm. for individuals from 10 to 17 years of age, and of 120 to 130 mm. for individuals from 21 to 40 years of age; Hirschfelder (1913) gives a maximum pressure of 110 to 135 mm. and a minimum pressure of 60 to 90 mm. for normal individuals at rest.

The conditions under which our observations were taken were so uniform that average or typical blood pressure curves, i.e., maximum, minimum, etc., could be made from the individual diurnal curves of the ten subjects. An examination of these average curves brings out two important points; first, the chief variations in the maximum blood pressure, mean pressure, pulse pressure, pulse rate, and relative velocity of the blood flow are all synchronous, and correspond to the periods of ingestion and digestion of food; second, minimum blood pressure varies little throughout the day, and there is no marked change corresponding to meals.

The average maximum blood pressure curve shows, after meals, a remarkably uniform rise of about 8 mm. of Hg above the pressure previous to the meal, i.e., after dinner on the first day 9.2 mm., after breakfast on the second day 7.8 mm., after luncheon 8.4 mm., after dinner 7.2 mm. The average rise of 8 mm. carries the pressure less than 5 mm. above the average maximum pressure of 120 mm. of Hg. The difference of 2 mm. of Hg in the height of the rise after dinner on the first and second days can probably be ascribed to psychical factors, since the

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subjects were temporarily in an unusual environment and were unfamiliar with the apparatus. We are aware, of course, that a difference of 2 mm. of Hg in blood pressure is ordinarily of no significance, but we are dealing here with the average of a considerable number of determinations so that slight variations become more significant.

After the 40 meals taken by the ten different individuals there is a rise in the maximum pressure in 38 cases; while in two cases there is no change. The amount of food taken or the time

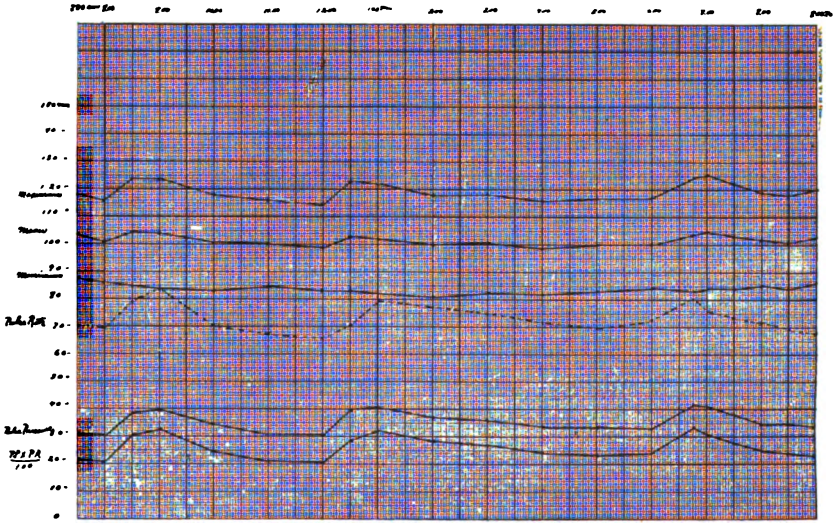


Plate 2. Average curves of the ten subjects for the main period of observation. The readings at 8.00 and 8.30 am., at 12.00 and 12.30, and at 6.00 and 6.45 p.m., were taken before and after meals, respectively.

of day, does not seem to have any effect upon the amount of the rise. The rise after dinner is no higher than that after luncheon and but 1 mm. higher than that after breakfast, although the evening meal was largest. A somewhat variable interval of time elapses before the maximum pressure approaches its former level: thus on the first day after dinner, two hours and three-quarters; on the second day after breakfast, two hours and a half; after luncheon it reaches its lowest point in three hours and a half, which is, however, 2 mm. higher than its former

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level; and after dinner, one hour and three-quarters. The difference of one hour in this time interval after dinner on the first and second days is rather striking and it seems probable that this may be caused by the psychical factors mentioned above. In general, an average of two and one-half hours elapse after a meal before the pressure approaches its former level.

We see from the average maximum pressure curve that as the pressure falls after meals it reaches the average of 120 mm. Hg in almost exactly one hour, and then continues to fall. This time interval would be a convenient one for clinicians and others to keep in mind in taking blood pressure readings, since at that time one may be reasonably sure of obtaining the average maximum pressure. But we must also take into account the possible range of variation in maximum pressure, thus, the greatest range in average maximum pressure as indicated by the plotted curve is about 13 mm.—the figures from which the curve was plotted being, of course, the averages for the several periods at which the pressure was read during the day. In individual cases, however, we find a variation in range of from 12 to 32 mm. for the entire period of the investigation—the specific ranges in mm. of Hg in the ten subjects being 12, 18 three times, 20, 22, 24, 26, and 32 twice. These ranges, then, may be considered possible for healthy young men. In cases in which we determined the average range for individuals, as we did for several, it appears that the extremes of maximum pressure are equidistant from the average maximum pressure. To cite one case: the average maximum for one subject is 127 mm., and his lowest and highest maxima are 116 and 138 mm. respectively.

The average minimum blood pressure curve varies little during the day, as we have noted above. The ingestion of food appears to have very little effect upon it, and when we examine the readings for the individual meals we find that the variation after meals is rarely more than 2 or 4 mm. above or below the reading before the meal, although occasionally it may be as much as 10 or 12 mm. of Hg. After the 40 meals taken by the ten different individuals there is a rise of the minimum pressure in 19 cases, a fall in 18 cases, and in three cases no change what-

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ever. In our average curve the lowest point is reached at 2 p.m., and this is only 4 mm. below the average of 85 mm. of Hg. The highest point recorded is the first reading in the morning, and the curve shows a gradual fall until 2 p.m., then a gradual rise until the last reading in the evening, which is nearly but not quite as high as that in the morning. The study of the figures taken in the individual cases shows that the individual curves are nearly as uniform as the average curve, but occasionally there are marked variations amounting to 10 or 12 mm. of Hg either up or down; from the records of the activities of the subjects the explanation for these variations is generally obvious. Thus, in one case in which the reading was 80 mm. at 8.30 p.m. it was 94 mm. at 9 p.m. just after the subject had undressed in a cold room. Again, a record of 92 mm. at 2 p.m. was followed by a record of 78 mm. at 3 p.m. the subject having been quietly reading during the hour.

In the pulse pressure curve there are the same variations corresponding to digestion that we find in the maximum pressure curve. The pulse pressure tends to increase throughout the day due to the general separation of the maximum and minimum pressures. Thus, before luncheon it is not quite as low as before breakfast, before dinner it is not quite as low as before luncheon, and at the last reading in the evening it is not quite as low as at the first record taken in the morning. The pulse pressure before dinner is practically the same on the first and second days, and during the second the increase in pressure due to meals is very uniformly about 9 mm. of Hg for each meal.

The typical pulse rate curve shows variations similar to those of the maximum and pulse pressure curves, occurring with the periods of digestion. There is an average increase of 10 beats per minute due to the meal. The increase at breakfast is most marked, probably due to the effects of the coffee taken. The increase at luncheon is greater than that at dinner. The maximum variation is about 18 beats, from 66 to 84 per minute, but in individual cases the range is, of course, greater. Thus in one case it was 42 beats, while in several it was from 26 to 28 beats. The increase in the average pulse rate after each meal does not

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seem to be proportional to the amount of food taken, since the height of the rise after breakfast, and after luncheon as well, is almost twice as great as that at the evening meal, although the former two meals were much smaller than dinner. After breakfast and luncheon the pulse rate does not reach its maximum until half an hour after the meal, while after dinner the highest rate is attained immediately after the meal. After the 40 meals taken by 10 different individuals there is an increase in the pulse rate in 36 cases, and a slight decrease in 4 cases. Of these 36 cases the increase begins immediately in 32, and in the remaining four it begins from one-half to three-quarters of an hour after the meal.

As might be expected, the relative velocity of the blood flow (PP $\times$ PR) increases after each meal. After luncheon it does not return to the rate before the meal, so that there is a gradual increase during the day.

To summarize briefly then, there are marked diurnal variations in maximum pressure, pulse pressure, and pulse rate immediately succeeding the ingestion of food. The maximum pressure is generally at its highest point immediately after the meal, and may remain at the same level for approximately half an hour. The pulse pressure, pulse rate, and velocity of the blood flow are increased immediately on the ingestion of food, but may not reach their maximum until half an hour later, after which they gradually decrease, and, except after the morning meal, do not fall to the level before the meal. There is, consequently, a gradual increase during the day. Erlanger and Hooker suggest that this gradual increase is due to the cumulative effect of the repeated ingestion of meals on digestion and metabolism. It seems probable, however, that the general increase in physical and psychical activities that occurs during the day may also be an important factor. It is evident that the ingestion and digestion of meals are the most important factors causing the marked diurnal variations in blood pressure in the normally active or resting individual.

Our results fail to show that the primary rise is followed by a secondary rise, as noted by Loeper 1912, although we made de-

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terminations after breakfast and luncheon at intervals of half an hour, and after dinner a second determination was made fifteen minutes after the first, and then every half hour.

A BRIEF REVIEW OF THE LITERATURE

Zadek (1881) made determinations with the Basch sphygmomanometer (which measures the maximum pressure) on three normal individuals. The observations were taken three times a day, i.e., morning, afternoon, and evening for periods of from three to nine successive days. He found that an average rise of from 8 to 15 mm. of Hg. occurred in the afternoon, and believed it to be entirely independent of the mid-day meal, which he observed caused a rise of from 10 to 20 mm. Toward night the pressure fell again, though it was still higher than in the morning. He found that the pulse rate varied inversely with the blood pressure.

Maximowitsch and Rieder (1890) made determinations with a Basch anaeroid sphygmomanometer on a few normal and pathological subjects to study the effects of the ingestion of liquids and muscular work upon the blood pressure. They found that when one-half a liter of water was taken a slight rise of pressure occurred followed by a gradual return to the original after 40 to 50 minutes. When greater quantities were taken, that is, one to two liters, drunk in 20 to 40 minutes, the return to normal did not occur until one to two hours later. Beer caused a more marked rise than water. One liter drunk within five minutes caused a rise of from 20 to 25 mm. of Hg, attaining its maximum in 50 minutes and returning to the original in one and a half hours. When greater quantities of beer were taken the rise remained from two to two and a half hours. The drinking of water as well as beer and other alcoholic beverages caused an increase in the pulse rate.

Howell (1897), while studying the relation of sleep to blood pressure by means of a plethysmograph, found that although there was a fall of pressure with the onset of sleep a gradual rise took place soon after until on awakening the pressure was the same as before sleep occurred.

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Hill (1898), while investigating, by means of the Hill-Barnard sphygmomanometer, the changes in the circulation caused by rest, sleep, and work, found the pressure as low when the subject was lying awake in bed in the morning as when lying awake in bed at night, and no lower when the subject was fast asleep. Observations, which he made hourly upon himself, show that his pressure was higher after a hard day's work than in the morning. He does not report the effect of meals upon the arterial pressure.

Colombo (1899), using a Mosso sphygmomanometer as well as that of Basch, reported observations on one normal individual although he mentioned having examined different persons. He determined the diurnal curve under two sets of conditions. First, meals, muscular exercise, and sleep were so arranged that they had no effect on the observations. This was done by changing the time of meals and waiting until their effects had presumably passed off before observations were taken, by refraining from muscular activity, and by changing the period of sleep. By taking observations during parts of several successive days a theoretical diurnal curve was made for one day. Second, meals, muscular exercise, and sleep were taken at the regular hours. The theoretical curve made with the Mosso sphygmomanometer, by means of the criterion of maximum oscillations shows that during the hours when digestion would ordinarily have taken place a fall of pressure occurred, although nothing had been eaten. During the morning hours before daybreak there was a rise of pressure although the subject was awake. During the warm hours of the day, from 2 to 4 p.m., the pressure was lower than during the cool hours of the forenoon, although the temperature of the laboratory did not change. The curve obtained with the Basch instrument, from the temporal artery by means of the criterion of the disappearance of the pulse palpated distal to the compression, showed changes in the pressure exactly the reverse of those obtained in the former case. When the Mosso apparatus was used with the criterion of the disappearance of the pulse, changes similar to those obtained with the Basch instrument were observed. When meals were taken the Mosso

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apparatus used with the criterion of maximum oscillations showed a fall in the blood pressure averaging 20 mm. of Hg, taking place immediately and attaining its lowest point two and a half hours later. This occurred whether there was a rise or a fall in the theoretical diurnal curve. The Basch instrument showed an elevation of pressure after meals and lasting four hours. An increase in the pulse rate, respiration, and body temperature also occurred after meals. Not recognizing that the Mosso apparatus as ordinarily used measures the minimum pressure, while the Basch instrument measures the maximum pressure, Colombo attempted to reconcile these apparently conflicting results by attributing the rise shown by the Basch instrument, when applied superficially to the temporal artery to the great vaso-dilation that occurs after meals. Because he believed the Mosso apparatus more sensitive he regarded the results obtained with it as the more accurate.

Weiss (1900) reported observations taken with Gärtner's tonometer on a normal individual. Thirteen readings were taken at intervals from 9 a.m. to 12 midnight on one day. After the morning and noon meals a marked fall in blood pressure occurred, while a rise took place after the evening meal. The final reading at night was 20 mm. of Hg higher than the first reading taken in the morning.

Hensen (1900) made determinations with a Riva-Rocci sphygmomanometer on a healthy girl who remained in bed for nineteen days. The observations were made twice a day, at 7 a.m. and 9 p.m. He found the evening readings from 5 to 15 mm. of Hg higher than the morning pressures. His chart showed that the pulse rate varied directly with the maximum pressure. Hensen believed with Zadek that there is a periodic afternoon rise independent of meals.

Brief reference may be made to Gumprecht (1900) who reported that he usually found a fall in pressure of 10 to 15 mm. of Hg in the forenoon, while the chief meal of the day generally caused a rise of about 20 mm. of Hg. He made the determinations with a Riva-Rocci sphygmomanometer.

Jellinek (1900) reported determinations made with Gärtner's

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tonometer on two healthy soldiers for a period of ten days, during which time they did only light sentry duty. The observations were taken six times a day, 5 a.m., 6 a.m., 11 a.m., 1 p.m., 5 p.m., and 8 p.m. He found the readings highest immediately after the noon meal and during the digestive period. The pressure was almost as high before the subject went to bed as in the morning. The pulse rate was not reported. He also made observations on twenty soldiers before and after meals, which consisted of soup, meat, vegetables, and bread. Of these subjects, fourteen showed a rise in pressure, two a fall, and four no change. The pulse rate increased in every case except one, in which a fall of blood pressure occurred and no change in the pulse rate. Jellinek concluded that the blood pressure was highest during the afternoon hours.

Brush and Fayerweather (1901) studied the changes which occur in blood pressure during the period of normal sleep. The observations were made with a hand plethysmograph used with the criterion of maximum oscillations. During the period of sleep the blood pressure in the wrist arteries fell for the first few hours, after which a gradual rise took place. Finally, on awakening in the morning the blood pressure was greater than before going to sleep in the late evening.

Brief mention may be made to Hayaski (1901), and Sommerfeld (1901), who found a rise of blood pressure after the midday meal.

Cook (1903) reported a rise of from 5 to 10 mm. of Hg in the maximum blood pressure of infants after feeding.

Goldwater (1903) using Gärtner's tonometer determined the blood pressure of a healthy medical student. He took readings nine times, from 9 a.m. to 10 p.m., during one day. The curve showed a continuous rise from 9 a.m. to 4 p.m., a slight fall from 4 p.m. to 6 p.m., and a marked rise after the evening meal until 10 p.m. On the next morning the pressure was the same as on the previous morning. He believed that there was a periodic rise in the afternoon which was independent of the midday meal. The fall of blood pressure during the digestive period, noted by Weiss (1900) who used Gärtner's tonometer, was not confirmed by Goldwater.

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Oliver (1903) published a chart of the arterial blood pressure taken with his haemodynamometer every hour from 8.30 a.m. to 10.30 p.m. A rise of blood pressure averaging from 15 to 20 mm. of Hg took place after meals, attaining its maximum in one hour and lasting from two and a half to four hours. The pressure at 10.30 p.m. was lower than at 8.30 p.m.

Karrenstein (1903), working with Gärtner's tonometer on soldiers, found a rise of from 10 to 20 mm. of Hg after the principal meal. There was always a rise of from 10 to 25 mm. after two or more liters of beer were drunk.

Janeway (1904) published a chart of the diurnal variations which he observed with the Janeway sphygmomanometer. He believed the curve to be a composite record of the effects of the various physical and mental states on the blood pressure. The chart showed a rise in maximum pressure on bathing and dressing in the morning, a fall after breakfast, a rise after the noon meal, a slight fall before the evening meal, a rise after it, and a fall before going to bed. The minimum pressure usually followed the maximum, except after the noon and evening meals when it fell. Janeway was inclined to believe that the maximum and minimum pressures were unequally affected by the taking in of food.

Erlanger and Hooker (1904), in a study of blood pressure and pulse pressure, described five experiments on an albuminuric patient, which showed variations in the maximum, minimum, and mean blood pressures, pulse pressure, pulse rate, and the product of the pulse pressure and the pulse rate. In each of the experiments the Erlanger sphygmomanometer was used and three readings made at each determination. In experiment 1 determinations were made every half hour from 7.35 a.m. to 10.30 p.m.; experiment 2, every half hour from 8 a.m. to 10 p.m.; experiment 3, every ten minutes from 9 a.m. to 2 p.m.; experiment 60, every few minutes from 9.15 a.m. to 3.55; experiment 65, every few minutes from 9.45 a.m. to 4.15 p.m.

Erlanger and Hooker believed it possible to explain diurnal variations almost entirely by the effect of the ingestion of meals. Experiments 1, 2, 60, and 65 brought out the fact that varia-

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tions in pulse pressure occurred in the form of waves corresponding to ingestion of meals. As a rule, there was an increase in the pulse pressure a few minutes after the ingestion of food, which reached its maximum in from one to two hours, and then declined more slowly, not wholly coming back to the level before the meal.

Experiments 2, 60, and 65 showed that when the pulse pressure reached its minimum it tended to increase very gradually until the next meal. There was a gradual increase in the pulse pressure throughout the day, upon which were built the wave-like increases that followed the ingestion of meals. There seemed to be no relation between the amount of food ingested and the increase in the pulse pressure. The increase after luncheon was greatest although there was no more food taken than at breakfast. The greatest amount of food was eaten at the evening meal but the increase in pressure was not as great as at luncheon. The maximum and minimum pressures tended to separate when food was taken; and, as a rule, the rise of the maximun was greater than the fall of the minimum, but not infrequently the reverse occurred. The mean blood pressure usually increased with the ingestion of meals, but often there was little or no change.

Experiments 2, 3, 60, and 65 showed that the pulse was accelerated with the pulse pressure. The product of the pulse pressure and pulse rate followed the pulse pressure curve. An increase in the relative velocity of the blood flow probably took place with the functional activity of the digestive organs.

Erlanger and Hooker believed that throughout the day the velocity of the blood flow increased, but that the gradual increase was partly obscured by waves of velocity changes induced by the ingestion of food. They attributed the gradual increase in the velocity of the blood flow to the cumulative effect of the repeated ingestion of meals on digestion and metabolism.

Brooks and Carrol (1912), in a clinical investigation of the effects of sleep and rest on blood pressure, studied a large number of pathological cases with the sphygmomanometers of Sahli and of Janeway, measuring only the maximum pressure. They considered the subjects in three groups, the first that of medium pressure, the second that of low pressure, and the third that of

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bigh pressure. Sixty-eight patients had an average afternoon pressure of 142 mm. of Hg. Two hours after the onset of sleep the pressure showed an average drop of 24 mm.; and three hours after awakening in the morning there was still a depression of 12 mm. Thirty-eight patients had an average afternoon pressure of 100 mm. of Hg. Two hours after the onset of sleep the pressure showed an average drop of 16.5 mm.; and three hours after the morning awakening there was still an average depression of 6.6 mm. Twenty-nine patients had an average afternoon pressure of 204 mm. Two hours after the onset of sleep the pressure showed an average fall of 44.8 mm.; and three hours after awakening in the morning there was still an average depression of 22.8 mm. The effect of food taken during the period of observation was not reported.

Loeper (1912) reported the determinations made with Pachon's sphygmomanometer on three normal individuals before and after a meal. In each case there was a primary rise occurring from 15 to 45 minutes after the meal, followed by a fall below the original. A second rise higher than the first occurred within three hours. He believed the first rise to be due to the distention of the stomach, and to be proportional to the amount of food taken. The first fall coincided with the period of gastric secretion, being more marked when irritating foods were taken. The second rise corresponded to the filling and distention of the intestine.

Faught (1913) published a chart of the variations of the maximum blood pressure during the working hours of a healthy young man. It showed that a rise in pressure took place after each meal, and a general rise during the day.

CONCLUSIONS

1. A rise of maximum pressure averaging 8 mm. of Hg occurs immediately on the ingestion of food. A gradual fall then takes place until the beginning of the next meal. There is also a slight general rise of the maximum pressure during the day.

2. The average maximum blood pressure for healthy young men in the neighborhood of 20 years of age is 120 mm. of Hg. This pressure obtains commonly one hour after meals. The

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higher maximum pressures occur immediately after meals, and the lower, as a rule, immediately before meals.

3. The range of maximum pressure varies considerably in different individuals, but the highest and lowest maximum pressures are practically equidistant from the average pressure of any one individual.

4. The minimum blood pressure is very uniform throughout the day, and is little affected by the ingestion and digestion of meals. When it is affected a rise or a fall may take place. There is a tendency for a slight general lowering of the minimum pressure throughout the day.

5. The average minimum blood pressure for healthy young men in the neighborhood of 20 years of age is 85 mm. of Hg. Thus we get an average pulse pressure of 35 mm. of Hg.

6. Pulse pressure, pulse rate, and the relative velocity of the blood flow are increased immediately upon the ingestion of meals. They attain the maximum, as a rule, in half an hour, and then decline slowly until the next meal. There is a general increase in each throughout the day.

7. The average pulse rate in these investigations proved to be 72 beats per minute.

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The average diurnal blood pressure record of the ten subjects

TIME	MAXIMUM	MINIMUM	MEAN	PULSE PRESSURE	PULSE RATE	PP × PR	NOTES
	mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg			
4.30 p.m.	119.5	84.1	101.8	35.4	72.0	2519	
5.00 p.m.	117.7	83.5	100.6	34.2	71.1	2432	
6.00 p.m.	118.0	84.0	101.0	34.0	74.9	2547	Before dinner
6.45 p.m.	127.2	88.2	107.7	39.0	78.1	3046	After dinner
7.00 p.m.	124.7	87.7	106.2	37.0	76.0	2812	
7.30 p.m.	122.0	83.4	102.7	38.6	76.0	2934	
8.00 p.m.	122.4	85.5	103.4	36.9	71.2	2527	
8.30 p.m.	120.0	85.0	102.5	35.0	69.7	2439	
9.00 p.m.	120.5	84.7	102.5	35.8	65.2	2334	
9.30 p.m.	118.2	84.4	101.6	33.8	64.4	2177	
7.30 a.m.	118.4	87.6	103.0	30.8	70.3	2165	
8.00 a.m.	116.4	86.4	101.4	30.0	69.8	2094	Before breakfast
8.30 a.m.	124.2	85.4	104.8	38.8	79.4	3081	After breakfast
9.00 a.m.	123.8	84.4	104.1	39.4	84.1	3313	
10.00 a.m.	118.2	83.6	100.9	34.6	70.7	2446	
11.00 a.m.	116.2	84.8	100.5	31.4	67.7	2126	
12.00 m.	114.4	83.2	98.8	31.2	66.2	2065	Before luncheon
12.30 p.m.	122.8	83.2	103.0	39.6	70.9	2808	After luncheon
1.00 p.m.	122.3	82.0	102.1	40.3	79.7	3212	
2.00 p.m.	118.4	81.4	99.9	37.0	77.6	2871	
3.00 p.m.	118.8	82.6	100.7	36.2	75.1	2719	
4.00 p.m.	115.8	82.0	98.9	33.8	71.9	2420	
5.00 p.m.	117.2	83.4	100.3	33.8	69.6	2352	
6.00 p.m.	117.4	84.4	100.9	33.0	72.8	2402	Before dinner
6.45 p.m.	124.6	83.1	103.8	41.5	80.4	3337	After dinner
7.00 p.m.	125.2	84.2	104.7	41.0	76.1	3120	
7.30 p.m.	122.0	84.0	103.0	38.0	73.7	2801	
8.00 p.m.	119.6	85.0	102.3	34.6	72.3	2502	
8.30 p.m.	119.7	84.0	101.3	34.7	69.0	2394	
9.00 p.m.	120.0	86.2	103.1	33.8	68.0	2298	
Average	120.0	85.0	102.5	35.0	72.0	2550	

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THE CONDITIONS OF CONDUCTION OF EXCITATION IN IRRITABLE CELLS AND TISSUES AND ESPECIALLY IN NERVE. II

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In its most general interpretation the "membrane-theory" of the bioelectric processes assumes that the electrical currents accompanying cell-activities such as stimulation, rhythmical activity, secretion, are mainly the expressions of variations in the electromotor properties of the surface-films or "plasma-membranes" bounding the living cells or irritable elements. In the original form of the theory as put forward by Bernstein in 1902, following a suggestion of Ostwald, the surface-film of the irritable element, e.g., muscle-cell, is regarded as "permeable" to certain cations contained within the cell, but not to other cations or to the generality of anions.[1] A selective permeability is thus assumed—equivalent to reversibility in the electrochemical sense—with reference to a particular cation, supposedly potassium; in consequence of this condition the membrane, so long as its normal resting semipermeability remains intact, is the seat of an electrical polarization similar to that at the surface of a metallic

[1] Bernstein (Archiv für die gesammte Physiologie, 1902, xcii, p. 521) interprets the conditions on the basis of diffusion-potentials, the membrane acting by decreasing the velocity of the anion; if this becomes zero the formula $E = \frac{u-v}{u+v} RT \ ln \frac{c_2}{c_1}$ becomes $E = RT \ ln \frac{c_2}{c_1}$, the same as the formula for electrode-potentials. The membrane, on this hypothesis, ought to act like a potassium electrode, but this appears not to be the case. It now seems probable that diffusion-potentials play a subordinate part, if any, in these phenomena. The following discussion therefore assumes merely that the demarcation-current potential, whatever its actual physico-chemical conditions may prove to be, depends for its existence on the semi-permeable condition of the membrane and diminishes or disappears when permeability is increased.

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plate; the layer of solution in contact with the cell-surface is positive relatively to the cell interior; in other words, the cell-surface behaves as if "reversible" to a particular cation just as (e.g.) a zinc plate is reversible to zinc ions. It thus becomes possible to conceive of the cell-surface as an electromotor surface having a certain definite ionic solution-tension. Theoretically the potential-difference across the surface should then have the value $E = RT\ln \frac{c_2}{c_1} + \text{const.}$, c_2 and c_1 being the respective concentrations of the reversible ion on the two sides of the surface.

Attempts to show that this relation holds for highly irritable living tissues like muscle and nerve have not met with entire success,[2] probably for the reason that the permeability, and hence the electromotor properties, of the cell-surfaces are in such tissues very sensitive to changes in the surroundings, and are hence readily altered by changes in the composition of the medium. With another and relatively resistant type of organic membrane a more satisfactory agreement with the demands of theory has been found by Loeb and Beutner.[3] The intact skin of the apple shows a behaviour indicating, within a considerable range of concentrations, a reversibility relatively to cations in general, and an indifference to anions; solutions of lecithin and fatty acids in organic solvents behave similarly. It seems hardly likely, however, that this condition holds for all of the cations—chiefly of alkali and alkali-earth salts—present in the cells and tissue-media of higher animals, for there is no evidence that the total concentration of these cations is higher within than without the cell, as the normal conditions in resting cells (with outer surface positive) would seem to require. I have suggested as a possibility a special reversibility to hydrogen-ions;[4] the H-ion concentration of the tissue-media is probably constantly lower than

[2] Macdonald's observations (Proceedings of Royal Society 1900, vol. lxvii, p. 325) appear to indicate for excised mammalian nerve a general reversibility to cations, as Loeb and Beutner have pointed out (Biochemische Zeitschrift, 1912, vol. xli, p. 3).

[3] Loeb and Beutner: Biochemische Zeitschrift, 1912, vol. xli, p. 1; ibid., xliv, p. 303; 1913, li, p. 288.

[4] American Journal of Physiology, 1908, vol. xxii, p. 86; 1911, xxviii, p. 206.

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that of the cell-interior, since the reaction of the former is automatically kept neutral, or slightly alkaline, and within the cell acids are constantly being produced by metabolism. The fact that variations in the external H-ion concentration have typically such marked influence on the activity and properties of irritable cells is in harmony with this assumption. It should also be noted that suspended cells like blood corpuscles show themselves normally negative in convection experiments and become indifferent at a certain definite external acidity[5]—another fact suggesting that the normal polarization depends largely on differences between outer and inner H-ion concentration. It is difficult to test this possibility for irritable tissues because of the quickness with which acid or alkali alters the state of the cell and renders conditions abnormal.

But whatever the nature of the ions concerned in these potentials may be, it is clear from the biological evidence that the normal electromotor properties of muscles or nerves are dependent on the intact condition of the semi-permeable membranes bounding the irritable elements. According to any theory of membrane-potentials, a difference in the electrolyte-content on the two sides of the membrane is essential; and a permanent difference in this respect between cell-interior and outer medium is possible only if the boundary-surface is impermeable to the electrolytes concerned in the production of the P.D. It is well-known that on death, or under the influence of substances or conditions that destroy semi-permeability, the demarcation-current potential diminishes or disappears. We may therefore hold with confidence that the normal electrical properties of irritable elements are a function of the condition of the limiting membranes, even though the exact nature of the membrane and the character and concentration of the ions concerned in producing the potentials are at present uncertain.

If we grant this, the conclusion is inevitable that any alteration in the permeability of the membrane to electrolytes must

[5] Cf. Michaëlis and Takahashi: Biochemische Zeitschrift, 1910, vol. 29, p. 439. Red blood-corpuscles become electrically indifferent at an H-ion concentration of *ca.* 1.25×10^{-5} normal.

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have electromotor effects; and there is ample evidence that the process of stimulation, which is always accompanied by bio-electric variations, is also constantly associated with increase in the permeability of the membranes. Artifically induced increase of permeability, by cytolytic substances and otherwise, always renders the irritable tissue negative.[6] The normal negative variation during stimulation may thus be explained as the expression of a temporary and reversible increase of permeability. If the membrane-theory is true, any loss of semi-permeability or of selective permeability to electrolytes would have the same effect as removal of the partition separating the intracellular and extracellular solutions; the P.D. would fall to what it would be if no such partition existed. The electromotor properties of the membrane in the excited region of a muscle-cell or nerve fibre must accordingly differ in a constant manner from those of the non-excited or resting regions. In the case of (e.g.) a nerve-axone which at one region is in a state of rest, at another in a state of excitation, we have a system which is similar—as regards the electrical effects produced in the system itself and in its surroundings—to a metallic wire differing in composition (and hence in solution-tension) at two different regions and immersed in an electrolyte-solution. In such a system the conditions for an electrical circuit exist, and a current flows along the wire and through the solution between the two regions. It is this kind of effect which in my former paper on the present subject[7] I have regarded as responsible for the transmission of the excitation-state from one region of the irritable element to the other. The current flowing between excited and unexcited regions arouses in the latter—through simple electrical stimulation—a

[6] That the application of cytolytic substances to an isoelectric muscle renders the tissue locally negative, and that this change is a delicate index of toxicity, was recognized by DuBois-Reymond: Untersuchungen über thierische Elektrizitat, vol. ii, 2d part, p. 159 *seq.* For further observations of this kind cf: Straub: Archiv f. exper. Path. u. Pharm., 1902, vol. xlviii, p. 1; Henze: Archiv f. d. ges. Physiol., 1902, xcii, p. 451; Allcock: Proc. Roy. Soc., B. 1906, lxxvii, p. 267; Straub: Zeitschr. f. Biol. 1912, lviii, p. 251; Hermanns: ibid., p. 261; Evans: ibid., 1913, lix, p. 397.

[7] Amer. Journ. Physiol., 1914, xxxiv, p. 414.

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second state of excitation, which itself serves as point of departure for a second current which similarly excites the resting axone at an equal distance beyond; this process automatically repeats itself, and in this manner the excitation-state is propagated along the fibre for an indefinite distance. In the present paper I propose to discuss in somewhat fuller detail, from a more purely electrochemical point of view, the nature of the conditions that render possible this form of transmission.

Let us first consider the case of a wire, e.g., of zinc, uniform in composition and immersed on a homogeneous solution of a zinc salt. The system is in electrical and chemical equilibrium, and no current flows along the wire. If now the concentration of the solution in contact with the wire is locally altered—by dilution or by adding more salt—an electromotor effect is at once produced, and a current flows along the wire from the more concentrated to the more diluted regions of the solution, and continues until the latter is again homogeneous. The case is that of a simple concentration-cell; the P.D. between the two regions is given by the formula $E = \frac{RT}{2F} ln \frac{c_2}{c_1}$, the more diluted region of solution being positive relatively to the more concentrated; zinc enters solution as zinc ions at the diluted region, and is deposited on the wire as metallic zinc at the more concentrated. The diagram (fig. 1) indicates the course of the current (positive stream); it is particularly to be noted that it enters the metallic surface from the solution at the more concentrated and leaves at the more dilute regions.

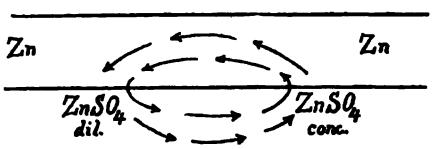


Fig. 1.

We shall now examine the question whether the bio-electric variation of stimulation can be regarded as the expression of a concentration-cell of essentially this type, i.e., one in which an electrolyte solution is in contact with an electromotor surface of unvarying properties, reversible with respect to certain cations present in the solution. In the case of a typical irritable element like a muscle-cell or nerve-axone the electromotor sur-

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face is represented by the protoplasmic surface-film or plasma-membrane; this membrane is in contact on both faces with an electrolyte solution; the external solution (e.g., lymph-plasma) is to be regarded as constant in composition; the internal solution (or cell-protoplasm) may vary locally in electrolyte-content in consequence of metabolic changes following stimulation. The substance of the membrane is reversible (i.e., permeable) relatively to the cations of an electrolyte produced or set free in metabolism. Let us suppose this variable electrolyte to be an acid, and the membrane-surface reversible to H-ions.[8] At the region of stimulation (*S*) acid, e.g., lactic acid, is produced; a concentration-cell effect at once appears, and a current flows within the cell and through the membrane and external medium in the direction indicated (fig. 2); the positive stream inside the cell will be *toward* the locus of increased acid-production, and away from it in the external part of the circuit. Part of the circuit is represented as passing through the external medium; this is possible because of the permeability (= reversibility) of the surface-film to the cations. The proportion of current thus traversing the unaltered regions of the cell at different points and returning through the surrounding medium will be determined simply by the relative conditions of electrical resistance in different parts of the circuit; an appreciable current may thus extend for a considerable distance from the altered region along the cell and into the surrounding medium, and may be detected externally by an electrometer.

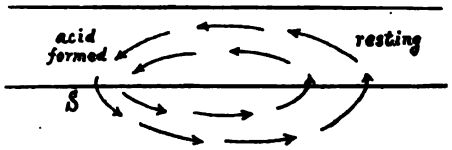


Fig. 2.

What should be noted especially is that in such a system the positive stream passes externally *from* the site of stimulation to the inactive region, i.e., in a direction the reverse of that observed in actual living tissues. We must therefore conclude that local production of acid or other active electrolyte in the neigh-

[8] I make this assumption provisionally, but it is justifiable for purposes of illustration, as at present.

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borhood of a semi-permeable membrane of unvarying properties, reversible relatively to the cations of the electrolyte, cannot possibly be the source of the action-current. This is still further indicated by the extreme variability in the time-relations of this current in different tissues and organisms, as set forth in my former paper;[9] these facts point quite clearly to determining conditions essentially different from diffusion; the interposition of changeable solid partitions is suggested. It seems advisable to emphasize these considerations here, since the view that local production of lactic acid is by itself sufficient to account for the characteristics of the action-current is still widespread among physiologists, although its inconsistency is easily demonstrated. If we assume that at the site of stimulation the electrolyte concerned in the production of the demarcation-current potential is suddenly *combined*, i.e., *decreased* in concentration, the external negativity of the stimulated area is theoretically intelligible. Otherwise—assuming reversibility of the electromotor surface to the cations alone (or chiefly)[10]—it is quite clear that electrolyte-production at the site of stimulation would cause externally a *positive* and not a negative variation.[11] We must therefore re-

[9] Loc. cit., p. 416 seq.

[10] If the electrolyte is an acid it is not necessary to assume any selective action of the membrane, since the H-ion has the greater mobility in any case.

[11] I have pointed this out briefly in an earlier paper (American Journal of Physiology, 1911, vol. xxviii, p. 208). Compare the remarks of Loeb and Beutner on the production of the injury-current (Biochemische Zeitschrift, 1912, vol. 44, p. 314); they also point out that local acid-production near an electromotor surface of this type would make the external surface of the element in this region *positive* relatively to unaltered regions. The hypothesis of Pauli (Kolloidchemie der Muskelkontraktion, Dresden, 1912, p. 6) assumes that acid *e.g.*, lactic, freed at the site of stimulation, is combined by protein, and that of the products of dissociation of this acid-protein complex only the anions are mobile; in effect this is equivalent to depriving the cations of their mobility. On the further assumption that the cell-surface is freely permeable to anions, it is possible to explain the fact that the variation is negative and not positive. A constant permeability to anions in the resting cell would however make the existence and characteristics of the demarcation-current potential quite unintelligible. The evidence indicates that the cell-surface allows anions to pass freely only during stimulation; this view however is quite distinct from Pauli's, and is discussed below. In Pauli's discussion the possibility that membranes play a part in the production of the bioelectric potentials is insufficiently considered.

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ject this conception of the physico-chemical conditions of the action-current as entirely inadequate.

The chief alternative view is that during stimulation the membrane or cell-surface undergoes a change in its electromotor properties, losing its selective reversibility (or permeability) to a single ion or class of ions and becoming more or less freely permeable to all. The stimulating agent or condition so alters the surface-film that it becomes temporarily permeable to anions as well as to cations; a reversible increase of permeability is assumed; this is the distinctive feature of the membrane-theory as first proposed by Bernstein,[12] and supported on various grounds by Brünings[13] and Hoeber,[14] and by myself in this country.[15] This theory postulates a change in the electromotor properties of the plasma-membrane during stimulation, and refers the electrical variation primarily to this, and not to any change in the intracellular electrolytes themselves. It should be noted that this view does not deny the possibility that such a change of electrolyte-content (e.g., acid-production) *may* occur as a secondary consequence of stimulation, and that in some tissues a part of the total electrical effect may be due to this.[16] The rapid and reversible electrical change in such a tissue as nerve is, however, considered to be essentially the expression of a similarly rapid and reversible alteration in the physical state of the colloidal surface-film of the irritable element. That such rapid changes in the properties of colloidal structures may occur in living tissues is indicated by the extreme quickness with which the tension of muscle-fibres and other colloidal structures like cilia may vary during contraction. Changes of permeability may presumably take place with at least the same rapidity as changes of mechanical tension.

[12] Bernstein: loc. cit.

[13] Brünings: Arch. f. d. ges. Physiol., 1903, xcviii, p. 241, and c, p. 367.

[14] Höber: cf. Physikalische Chemie der Zelle und der Gewebe, 4th ed., 1914, chapter 12, p. 579.

[15] Cf. Amer. Journ. Physiol., 1908, xxii, p. 75; 1909, xxiv, p. 14; 1911, xxviii, p. 197.

[16] This is possibly the case in muscle, where the E. M. F. of the action-current is said at times to exceed that of the demarcation-current. Cf. my note in Science, N. S., 1912, xxxvi, p. 437.

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Let us now briefly consider some of the presuppositions and consequences of this hypothesis. Evidence of increased permeability of the plasma-membranes during stimulation, as well as during analogous changes like the activation of the resting egg, comes from many sides.[17] This evidence, it should be noted, is quite independent of any hypotheses as to the physico-chemical conditions of the action-current. Changes of turgor, loss of substance from the cell, increased penetration of substances from outside, and increased electrical conductivity have all been observed in association with different forms of stimulation or activation; here we have simply facts of observation. These observations have not all been made on the same type of cell: increased conductivity and entrance of outside substances have been demonstrated with certainty only for egg-cells;[18] and some physiologists may hesitate to regard the activation of such cells as a case of stimulation; but the analogies[19] are close, and such facts must be considered in forming a general conception of the stimulation process. The fact that cytolytic or permeability-increasing agents have in general a stimulating action should also be noted;[20] substances not so acting (like sugars, or salts in physiologically balanced proportions) are typically indifferent in their action on irritable elements. There is thus a large body of purely observational evidence indicating that increase of surface-permeability is a constant accompaniment of stimulation. Reasoning of a more purely theoretical kind supports this conclusion. Any general increase in the permeability of a partition separating two electrolyte-solutions must alter the electromotor properties

[17] For a general account of this evidence cf. my articles in the Popular Science Monthly, 1913, p. 132, and 1914, p. 579; also Amer. Journ. Physiol., loc. cit., 1909, 1911.

[18] For increased conductivity of egg-cells following fertilization cf. McClendon: Amer. Journ. Physiol., 1910, xxvii, p. 240; Gray: Journal of the Marine Biological Association, 1913, x, p. 50. For evidence of increased permeability to dissolved substances cf. Lyon and Shackell: Science, N. S. 1910, xxxii, p. 249; Harvey: ibid., p. 565; also Journ. Exper. Zoology, 1911, x, p. 547.

[19] These analogies are discussed at greater length in my paper in Journ. Exper. Zoology, 1913, xv, p. 23.

[20] For experimental data of this kind cf. my paper in Amer. Journ. Physiol., 1911, xxviii, p. 214.

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of the partition, since the two solutions are then less effectually separated than before and tend to approach each other in composition. We find in fact that in irritable tissues like muscle and nerve a demarcation-current is produced by any cytolytic substance, or by injury of the surface through any means, mechanical or otherwise;[21] this current is undoubtedly due to the increase of surface-permeability at the region acted upon; the effect is the same as that associated with natural death of the tissue, a change always involving loss of semi-permeability. It is thus significant that the electromotor variation accompanying stimulation is similar both in direction and order of dimensions to the demarcation-current, differing only in its time-relations and reversibility. The assumption that a similar increase of permeability, only rapid and completely reversible in character, takes place in the surface-film of the irritable element, is therefore sufficient to account for the main characteristics of the bio-electric variation of stimulation. The rate and degree of this change of permeability, and also the readiness with which it occurs under changes of condition, must be regarded as varying from tissue to tissue; the specific differences in irritability and in rate of response may be thus explained. According to this view, the time-relations of the bio-electric variation in any tissue correspond with those of the associated variation of permeability.

We shall now consider the nature of the electrical effects resulting from a change in the electromotor properties of the plasma-membrane of the kind indicated above. The conditions may again be best understood by comparison with a simple inorganic model (fig. 3). Let us take the case of a piece of galvanized iron wire, i.e., iron core covered by a thin layer of pure zinc, immersed in a dilute solution of sulphuric acid. If the surface-film of zinc is homogeneous, no effect, chemical or electric,

[21] Cf. footnote 6. Loeb and Beutner (Biochemische Zeitschrift, 1912, vol. xliv, p. 303) find that local injury (as crushing) to apple-skin (a membrane reversible to cations as a class and indifferent to anions) modifies the electromotor behavior of the membrane, making it negative relatively to uninjured regions. It is interesting to note that at the same time the general permeability is increased; the readier entrance of oxygen (e.g.) is shown by the browning of the pulp at the injured region.

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is seen. If however the wire is bent or otherwise altered at any region, *S*, so as to interrupt the continuity of the outer layer and expose the underlying iron, both chemical and electrical effects at once appear. There are now two electro-motor surfaces of unequal solution-tension in contact with the solution; zinc ions accordingly enter into solution from the zinc surface, and H-ions are deionized and appear as hydrogen gas at the iron surface, while an electrical current flows in the wire from iron to zinc as indicated.[22]

The manner in which the current distributes itself through the circuit consisting of the wire and the surrounding solution should be noted. It is not confined to the immediate neighborhood of the region where both metals are exposed side by side (*S*), but extends for an indefinite distance on either side of this and through the medium in the manner indicated. The strength of the current is naturally greatest near the junction *S*; the relative strengths of current flowing through different sections of the wire at different distances from *S* (*SA*, *SB*, *SC*, etc.) are dependent on the relative resistances of the respective circuits consisting of the stretch of wire *SA* (etc.) and the intervening solution. In other words, the distribution

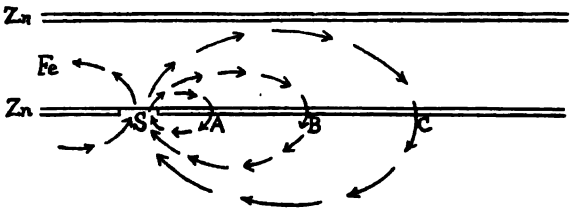


Fig. 3.

[22] In the case of the rhythmical hydrogen peroxide catalysis of Bredig, the current assumed by Antropoff (Zeitschr. f. physik. Chem., 1907, vol. lxii, p. 513) to flow between the film-covered mercury-surface and the free metallic surface (when the latter is exposed by rupture of the film) originates in an essentially similar manner, as I pointed out in my last paper (p. 426). Also the electromotor effects described by Bose (Response in the Living and Non-living, 1902, and Electrophysiology, 1907) as resulting from the bending of wires of tin and other metals, and compared by him to bioelectric currents, belong in the same class of phenomena. The reason why they show such striking analogies with the injury-currents or action-currents of living tissues will be evident from the above considerations. In both cases changes in the electromotor properties of surfaces are concerned.

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of current-intensities at different points of the circuit is determined by Ohm's law; in a cylindrical wire the intensity is thus at any point inversely proportional to the distance from the junction of the two surfaces.

The case of the living cell (e.g., muscle-cell) or nerve-axone is to be regarded from an essentially similar point of view. The assumptions as to the precise nature of the conditions need not be too detailed. We observe that a current flows externally from uninjured to injured, or from unstimulated to stimulated regions of the living element. We have only to assume that the injured or stimulated protoplasmic surface differs in a constant manner in electromotive properties from the normal resting surface in order to account for the essential phenomena observed. The evidence[23] indicates that the normal unaltered surface-layer of the irritable element acts as an electromotor surface reversible to—or permeable to—certain cations only, but that the altered or stimulated surface is permeable to ions of both classes. This general view need not necessarily subscribe to Bernstein's original theory that the observed potentials are simple diffusion-potentials, modified by interposition of a chemically indifferent membrane having selective physical permeability to certain cations. The reversibility of the electromotor surface to cations, which it seems necessary to assume, may depend on quite other conditions, as the very interesting investigations of Beutner[24] appear to render highly probable. For our present purpose all that is necessary to assume is (1) that the plasma-membrane of the irritable element during rest forms an electromotor surface reversible with reference to certain cations which are present in the cell in higher concentration than in the outer medium; and (2) that during stimulation the membrane becomes freely permeable to certain anions as well. The P.D. between the cell and the outer medium is thus suddenly lowered at the site of

[23] I.e., external positivity, and increase of positivity with decrease in the concentration of the outer electrolyte.

[24] Cf. Beutner: Journ. Amer. Chem. Soc., 1913, xxxv, p. 344; Zeitschr. f. Elektrochemie, 1913, xix, pp. 319, 467; Trans. Amer. Electrochem. Soc., 1913, xxiii, p. 401; Amer. Journ. Physiol., 1913, xxxi, p. 343.

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stimulation. The conditions are then, as regards flow of current, the same as if the region of stimulation were a region of lower solution-tension (relatively to cations) than the resting region. The resemblance to the above model thus becomes evident (see fig. 4).

During the resting condition cations are unable to leave the cell because of the impermeability of the surface-film to anions and the resulting electrostatic stress; the normal polarized condition of the resting element (with outer surface positive) is thus accounted for. If then at any point in the surface the membrane is altered by stimulation so as to allow anions to leave the cell, the electrostatic tension holding back cations is at once released —not only at the region of stimulation, but at all other points of the cell-surface; cations are then free to leave the cell in numbers equivalent to the anions: in other words, a current then flows between unaltered and altered regions as represented in the diagram, i.e., from resting to stimulated region (S) in the external part of the circuit, and in the reverse direction within the cell.[25] The intensity of this current will differ in different parts of the circuit; in the interior of the cell it will be greatest near the site of alteration (S), just as in the inorganic model, decreasing as the distance from this point increases. The conditions as regards distribution of current-intensities are in fact precisely analogous to those indicated above for the model; i.e., the current flowing lengthwise in the interior of the element away from the altered region (i.e., in the intracellular part of the circuit)

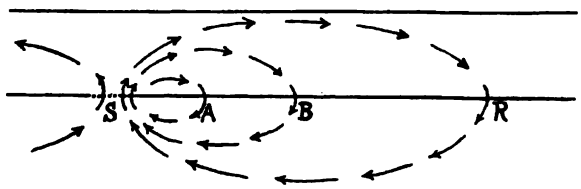


Fig. 4.

[25] This circuit may be called for brevity the "stimulated-resting circuit"—or perhaps better the "active-inactive circuit," since this expression is applicable to automatic tissues like heart-muscle as well as to those requiring external stimuli. I shall use the term "active-inactive circuit" in the remainder of this paper.

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decreases in intensity in direct proportion to the distance from the site of stimulation. In the surrounding medium there is of course an opposite or "return" current (extracellular part of active-inactive circuit) whose intensity at any region is similarly determined by conditions of resistance.

It is important to note that the changes in electrical tension due to the alteration of the membrane are transmitted *instantaneously*[26] to all points of the circuit. Hence simultaneously with the electromotor variation at region S there is initiated in the external part of the circuit, and hence at all points in the layer of solution in contact with the external surface of the membrane, a movement of cations toward S; similarly in the layer of solution adjoining the inner surface of the membrane there is a movement of cations in the opposite direction:[27] the effect of these movements at any point of the membrane is to *diminish* the potential-difference between its opposite faces, i.e., to cause a depolarization-effect of a greater or lesser degree.[28]

[26] *I.e.*, if electrostatic retardation and self-induction are inappreciable, with the velocity of light. At the risk of appearing to insist needlessly on elementary points of theory, it seems well to remind biological readers that the velocity with which an electrical current or a change in the intensity of a current is transmitted through a circuit is the velocity with which electrical tensions or changes of tension are transmitted, i.e., the impulse which sets the ions or electrons in motion—which is quite independent of the velocity of the individual ions or electrons themselves. This last velocity, as well known, is very slow (measured in centimeters per hour per volt-centimeter for ions in solution).

[27] Anions, if free to move, will travel in the opposite direction to cations; according to the present hypothesis, however, anions can traverse the surface of the element only at S (the region in a state of excitation); for every unaccompanied anion leaving the element in this region, an equivalent cation must be assumed to leave at the unaltered region beyond S.

[28] That is, the direction of the current in the active-inactive circuit, where it passes the membrane in the resting region, is opposed to the normal polarization of the membrane (the latter being positive externally; negative internally). Compensation or depolarization thus results here to a greater or less degree, depending on the distance from S. It should be noted that this conclusion is independent of deduction from the present theory; the observed direction of the action-current in the external part of the circuit shows that it flows in fact in the course indicated, whatever its mode of origin may be. Hence when it traverses a surface polarized in the sense of the plasma membrane, the potential between the opposite faces of the latter is necessarily decreased; i.e., the current exerts a depolarizing action, which is equivalent to a stimulating action.

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This consequence is of the greatest importance in the present theory of conduction; because it is precisely such depolarizing effects which underlie the general stimulating action of an electrical current, as the law of polar stimulation clearly indicates. The current traversing the irritable element between the altered and unaltered regions has therefore *such a direction that it tends to stimulate the resting portions of the element* (see fig. 5). Whether stimulation at any point distant from S actually results or not depends (1) on the distance of that point from the region of alteration—since it is upon this that the intensity of the current traversing that part of the circuit depends—and (2) on the sensitivity of the element to electrical stimulation.

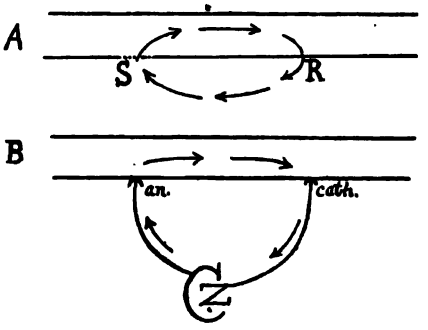


Fig. 5. In *A* the arrows represent the direction of the current in the active-inactive circuit on one side of the stimulated region *S*. In *B* the course of an external stimulating current from a battery is represented. Stimulation originates, on make, at the cathodal region, where the current has the same direction, relatively to the membrane, as at *R*.

In general therefore we conclude that in any irritable element like a nerve-axone the effect of a sudden alteration of the membrane at any region S is to produce at neighboring unaltered regions an immediate depolarizing effect, which at all points (B, C, etc.) within a certain maximal distance SR (beyond which the current-intensity is too weak) will have a stimulating action. Since this secondary stimulation involves the same alteration of the membrane as at the original point S, and since the all-or-none law holds for the nerve-axone, it is clear that the most distant (from S) part of the secondarily excited region will at once become the point of departure for a second electrical impulse similar to the first. This will in a similar manner induce excitation at a similar distance beyond. According to the present theory, the spread of the excitation-state from the original point of stimulation depends on these conditions. I need not repeat here the exposition given in my former paper.

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It is clear from what has been said that the velocity of such a transmission will be a function of the *rate* at which the membrane undergoes its characteristic electromotor change, and of the sensitivity of the element to electrical stimulation. There is evidence that with a high degree of sensitivity, as in nerve, the electrical impulse arising from the local alteration may suffice to set in motion the excitation process in the adjoining regions at all points up to a distance of two or three centimeters from the original site of stimulation.[29] Since the purely electrical transmission is instantaneous, the excitation-process at any point in this second region will begin at the instant[30] the current passing across the membrane (between S and R) has attained a sufficient intensity and duration.[31] Excitation is thus initiated on both sides of region S up to a distance SR, beyond which the current is ineffective.

The interval between the beginning of excitation at S and that of the secondary excitation at R will depend on the rate at which the electromotor variation at S develops, and on the latent period of excitation of the irritable element; this last interval, however, is very brief in a rapidly responding tissue like nerve, and may be neglected for simplification. The flow of current through the unexcited region SR begins simultaneously with the electromotor change of the membrane at the excited region S, and at any point follows a curve which parallels in time-relations and variations of intensity the curve of the electromotor change at S. A certain time must elapse before this current begins to have stimulating action at any point in the region SR. Presumably the time required for the transmission of excitation from S to R will be a certain constant ratio of the duration of the electromotor variation at S. According to the general rules governing electrical excitation, the current passing across the membrane at R must have a certain duration (t) and intensity (i) before it begins to excite. Exactly what those are need not be consid-

[29] Cf. my earlier paper, loc. cit., 1914, p. 431. seq.

[30] Allowing for the latent period of excitation, which in nerve is a small fraction of the whole duration of the excitation-process.

[31] Its rate of change is amply sufficient, as pointed out in my former paper (p. 438).

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ered at present; it is sufficient to note that at the farthest point thus secondarily stimulated (R) the product $i\sqrt{t}$ will have the minimal value that is effective.[32] In my former paper I have regarded the time required for the development of the necessary current at R as equal to that occupied by the rising phase of the electromotor variation at S (i.e., about half the total duration of this variation at any point).[33] The excitation-process aroused after this interval (*ca.* 0.001 second in nerve at 20°) at R then pursues a course similar to the original one at S, and transmits in a similar time its effects to further portions of the element. In order to account for the nervous transmission-velocity of 30 meters per second in frog's motor nerve at 20°, the distance SR would have to be 3 cm., on the assumption that the rise of the bioelectric variation at any point occupies 0.001 second and (for simplification) that the position of S remains stationary during the transmission from S to R.[34] Evidence that currents

[32] Assuming the validity of Nernst's rule, that stimulating effect (like polarizing effect), S, equals $Ki\sqrt{t}$, $-K$ being the coefficient of irritability (or irritability-constant) of the element. Since the "all-or-none" law holds for nerve, there are no differences in the effectiveness of the stimulation at R and at other regions (A, B, etc.) nearer S. R is thus the only point to be considered in estimating the rate at which the excitation spreads.

[33] It may be less, but hardly more than this; the maximal intensity of the current across R is reached when the curve of electromotor variation at S is at its apex, but this intensity lasts for a short time only. An intensity of two-thirds this value (or more) will however last for a considerable fraction of the time occupied by the whole variation; to know just what this fraction is, it would be necessary to determine more accurately the form and time-relations of the curve of electromotor variation at S; the fraction would then be the ratio of the horizontal line between opposite slopes of the curve, at two-thirds the height of its axis, to the base-line. The stimulating action of a current is measured by $Ki\sqrt{t}$; at a certain maximal distance beyond S the current, though weaker than at nearer points (A, B, etc.) ,will still have an intensity and duration sufficient for excitation. It should be remembered that the *whole* intermediate region between S and R is excited; R is merely the farthest point to which excitation extends. The influence of this intermediate region SR, any point in which will be excited sooner than R, must enter in the transmission. Needless to say, the latter is continuous and not by leaps.

[34] This assumption simplifies without essentially altering this phase of the problem. In reality S is moving forward at a rapid rate (30 m.-sec.). If this fact is also considered, the assumed maximal distance through which the electromotor variation at S exerts effective action might be considerably less than

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similar in electromotive force to the action-current can stimulate a nerve when a portion of the latter 2 to 3 cm. in length is interposed in the circuit is contained in my former paper.[35]

All that is required by the present theory of conduction, in its application to rapidly conducting tissues like nerve or voluntary muscle, is that the electrical current resulting from the electromotor variation of the membrane at the site of stimulation should make its effect felt as stimulus for a considerable distance (probably 2 to 3 cm. in nerve) beyond the region which is actually undergoing the changes associated with stimulation. A certain degree of electrical conductivity in the tissue and adjoining medium is thus presupposed; the necessity for salts in the medium, as well as in the cell itself, is probably in large part an expression of this requirement. One long recognized structural peculiarity of rapidly conducting tissues is the elongated character of the elements, i.e., the existence of protoplasmic continuity, without cross-partitions, through relatively great distances. This condition is especially characteristic of nerve fibres and voluntary muscle-fibres; in such a tissue as mammalian heart muscle, where the elements are relatively short, the protoplasm is apparently continuous from cell to cell; this condition is usually regarded as a special device for promoting rapidity of conduction. A similar continuity is found in the nerve nets of medusae. It is well known however that one tissue may be excited by the action-current of another, so that protoplasmic continuity is not necessary, though it may be favorable, to this type of transmission. Its existence in tissues with highly developed power of conduction is evidence of such favorability, and the reason for this becomes clear on the present theory. The electrical resistance of unaltered plasma-membranes is high; evidently, therefore, the interposition of a membranous partition in a circuit part of which is formed by the locally stimulated element would greatly diminish the intensity, and hence the stimulating action, of the intracellular current at points beyond the partition. The absence of

3 cm. and still be sufficient to account for the propagation-velocity of the wave of excitation.

[35] Cf. p. 431. seq.

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cross partitions in nerve-axones thus means that electrical conductivity along the fibre is as high as conditions permit; the current arising from any local alteration is thus effective through the greatest possible distance from the point of stimulation.[36] It is known that both nerve and voluntary muscle exhibit a much greater electrical conductivity (per unit of length and sectional area) in a lengthwise than in a transverse direction.[37] The high resistance in the latter case is undoubtedly due to the membranes interposed in the path of the current at the boundaries of cells and sheaths; in dead tissues the difference between lengthwise and transverse conductivities is decreased or disappears.

In medullated nerve the structural arrangements are peculiar; both axone and medullary sheath, being cellular elements, are to be regarded as limited externally by semi-permeable membranes; the enclosure of the axone by a concentric sheath of this nature will thus have the effect of preventing the current in the extracellular part of the active-inactive circuit from spreading beyond the boundary-membrane of the sheath and exciting the axones of adjoining fibres. The conception of the medullary sheath as an insulating layer in this latter sense is thus consistent with the present theory; the structural conditions are such that the path of the return-current of the active-inactive circuit is confined within definite limits. The insulation of conducting paths is thus rendered possible in this tissue to a greater degree than in any other.

As a general theory of physiological conduction, the present

[36] No Paleyism is intended here. The conductivity of an electrolytic conductor of the physico-chemical constitution of living protoplasm is limited by the necessary conditions of its composition and structure. Given a cylindrical element of this constitution, its conductivity is as great as possible if cross-partitions are absent. It may be noted that the greater longitudinal conductivity of nerve and muscle-cells is evidence that the inner conductivity of the cell is much greater than that of its surface-film.

[37] Cf. Hermann: Arch. f. d. ges. Physiol., 1872, v, p. 223. Macdonald's observations on the differences in the resistance exhibited by a given stretch of nerve according to the position of the electrodes—resistance being higher when the latter are applied at longitudinal than at cross-sections of the nerve—illustrate the same phenomenon (Proc. Roy. Soc. 1900, lxvii, p. 310).

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view meets with the difficulty that local mechanical injury or poisoning destroys the physiological conductivity of a nerve or other conducting tissue without interfering with its electrical conductivity. This fact appears at first sight inconsistent with the view that conduction of stimuli depends on a purely electrical transmission of the kind above described. In reply to this objection it was pointed out in my former paper that any such local alteration inevitably gives rise to a current of injury, and that quite possibly this current, having an opposite direction to the current in the approaching active-inactive circuit, might compensate the latter and so annul its effect. In further support of this hypothesis the mutual interference of two excitation-waves which meet each other in the same tissue may be cited. This phenomenon is seen when a stimulus is applied at one point in a ring-shaped piece of conducting tissues; such rings may readily be cut from the subumbrella of a medusa.[38] Two excitation-waves (whose course is shown by the associated muscular contraction) travel in opposite directions from the point of stimulation and meet on the opposite side of the ring; there, if equal in size, they at once extinguish each other. This case appears to be identical in principle with the extinction of an excitation-wave on entering a region traversed by a demarcation-current. In such a nerve-ring we may for simplicity regard the one region of excitation as stationary; the tissue adjoining this region is traversed by an active-inactive circuit with a definite orientation. In the approaching second region of excitation there is a similar but oppositely orientated electrical circuit. The two on meeting undergo mutual compensation; excitation is accordingly arrested at the region of intersection, since the transmission to further regions depends on the existence of the current. On any other hypothesis this interference seems difficult to explain; while accordingly to the purely physical rules of compensation two such similar and oppositely orientated currents meeting each other as indicated would certainly undergo mutual extinction; accordingly the fact that excitation is extinguished by such in-

[38] Cf. A. G. Mayer: "Rhythmical Pulsation in Scyphomedusæ," Carnegie Institution Publication, No. 102, 1908, p. 116.

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tersection strongly favors the view that the electrical accompaniment of the excitation-process is the actual basis of its transmission from region to region. Obviously it is a corollary of the present view that extinction of the electrical variation by compensation necessitates extinction of the excitation-wave.

The foregoing view of the mechanism of conduction has certain general implications with regard to the nature of the conditions controlling the normal activity of living cells. In particular it emphasizes the importance of electrical factors in the processes underlying the response to stimulation. What seems peculiar to the irritable element is its property of exhibiting a definite change of activity—or "response"—whenever the electrical polarization of its boundary-surface is suddenly altered (typically decreased). This alteration may result from any sufficient mechanical or chemical change in the membrane, or from the action of an external current. It seems necessary to assume further that this polarization-change initiates in the living system a local chemical reaction, as one result of which the membrane loses temporarily its normal electromotor properties and semi-permeability; this change involves further electrical effects of the kind already described, and through the influence of the local circuit thus arising the effect spreads. The direction of the current in this circuit (active-inactive circuit) explains why in any irritable element the effect of local excitation cannot be confined to the region directly affected; the automatic tendency for the excitation-state to spread is inherent in the nature of the mechanism itself. Hence no essential distinction can be drawn between the local excitation of an irritable element and the conduction of the excitation-state to adjoining regions.

A high degree of irritability thus corresponds to a condition in which the cell or irritable element is especially sensitive to changes in the electrical polarization of its surface-film. All of the facts of electrical stimulation indicate that any such change, if sufficient in rate and intensity, initiates some metabolic reaction, possibly oxidation, which involves alteration of the plasma-membrane in the manner indicated. This property evidently depends on a somewhat delicate balance of conditions in the living

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system; and in fact it may readily be modified in a reversible manner; for example, the membrane may be made more resistant than normally to changes of permeability, and then alterations in its polarization may have no effect; apparently this is the kind of modification induced by anaesthetics and certain salts like magnesium chloride.[39] Hence such substances abolish irritability and its correlate conductivity. That metabolic processes underlie the surface-changes of stimulation is shown clearly by the influence of temperature on the rate of excitation and conduction in normal tissues; but the precise nature of the purely chemical factor in these changes is unknown and can be determined only by further experimental investigation.

SUMMARY

1. It is shown that if the result of local stimulation of an irritable cell or element (e.g., nerve-axone) is to change the electromotor properties of the surface-film in such a manner as to abolish its characteristic selective reversibility to cations, an electrical current at once flows in the circuit: from active region to inactive region to external medium, and back to active region. This circuit may be called the "active-inactive circuit."

2. This current flows within the cell or axone (i.e., in the intracellular part of the circuit) from active toward inactive regions, and in the external medium in the reverse direction. It has therefore such a direction as to lessen or partly compensate the normal electrical surface-polarization (outer surface positive) in the still inactive regions of the irritable element.

3. This depolarization results in stimulation at all points not too far removed from the original site of alteration. The transmission of the excitation-state from active to inactive regions depends on this condition. The rate of propagation depends on two main factors: (1) the rate at which the electromotor variation develops at the region of excitation, and (2) the maximal distance through which the current in the active-inactive circuit

[39] Cf. my papers on antagonism between salts and anaesthetics: Amer. Journ. Physiol., 1912, xxix, p. 372 and 1913, xxxi, p. 255.

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is able to exert stimulating action. This rate is estimated by the quotient s/t, t being the time required for the local electromotor variation to develop to a certain critical point, and s the maximal distance through which the current is effective as stimulus. In any special case (like nerve) it is to be presumed that s is a constant fraction of the total duration of the local variation.

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THE ANALYSIS OF NITROUS OXIDE FOR PHYSIOLOGICAL WORK

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For various problems in physiology, especially that of the measurement of the blood flow through the lungs of man, as described by Krogh and Lindhard, it is necessary to analyze with great accuracy gas mixtures containing nitrous oxide. Krogh and Lindhard[1] in their paper on the blood flow describe a method of combustion of nitrous oxide with hydrogen, using a modified Haldane gas analysis apparatus.[2] Further details are given in subsequent papers by Krogh[3] and by Lindhard.[4] Their method is much simpler and more rapid than that described by Markoff, Müller, and Zuntz.[5]

Various text-books on gas analysis refer to the combustion method of nitrous oxide but do not give details by which it may be carried out, and in fact usually give the impression that the method is not one of great accuracy.

[1] Krogh and Lindhard: Measurement of the blood flow through the lungs of man. Skand. Archiv. f. Physiol., 1912, xxvii, p. 100.

[2] Haldane: Methods of air analysis. 1912, Griffin and Co., London.

[3] Krogh: Funktionsuntersuchungen an den Lungen des Menschen mittelst gasanalytischer Methoden; Abderhalden: Handbuch der Biochem. Arbeitsmethoden, 1915, Urban and Schwarzenberg, pp. 550.

[4] Lindhard: Concerning the influence of ultraviolet light on the circulation in man. Skand. Archiv. f. Physiol., 1913, xxx, 73–96; Lindhard: Effect of posture on the output of the heart. Skand. Archiv. f. Physiol., 1913, xxx, 395–408.

[5] Markoff, Müller, and Zuntz: Eine Stickoxydul-Methode zur Bestimmung der umlaufenden Blutmenge in lebenden Korper. Ztschft. f. Balneologie, 1912, iv, 14–15.

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In light of the article by Baskerville and Stevenson[6] on the analysis of nitrous oxide in which, on the authority of Lunge,[7] the combustion of nitrous oxide with hydrogen is stated to be unreliable, we determined to reinvestigate this point before proceeding to the use of the method in physiological work.

Deviation from the gas laws. Nitrous oxide is not a perfect gas and its deviation from the gas laws is considerable. At the usual temperatures and pressures existing in a laboratory it is of the order of 0.7 per cent. However, in the method of analysis by which nitrous oxide is combusted with hydrogen to form nitrogen and water and the contraction measured, the above deviation is automatically compensated as the following consideration shows. The litre weight of nitrous oxide at 0° and 760 mm., were it a perfect gas, would equal

$$\frac{\text{Molecular weight of nitrous oxide}}{\text{Molecular volume}} = \frac{44.014}{22.412} = 1.964 \text{ grams}$$

But the litre weight at 0° and 760 mm. is actually found to be 1.9777 grams.[8] That is, at 0° and 760 mm. the deviation of nitrous oxide from the gas laws is $\frac{1.9777 - 1.964}{1.964} = 0.7$ per cent.

This error need not be taken into consideration in the analysis as shown by the following calculation. Let N be the number of molecules of hydrogen or nitrogen per cubic centimeter at the constant conditions of the experiment. Hydrogen is added in excess to 1 cc. of nitrous oxide. At 20° and 760 mm., 1 litre of nitrous oxide contains about 1.006 times as many molecules as nitrogen or hydrogen under the same conditions. In this case, 1.006 molecules of nitrous oxide must be decomposed, and therefore 1.006 molecules of hydrogen are required since

$$N_2O + H_2 \rightleftarrows N_2 + H_2O$$

and as hydrogen is nearly "perfect" under these conditions, 1.006 cc. of hydrogen will be used up. But at the same time 1.006

[6] Baskerville and Stevenson: Contributions to the chemistry of anaesthetics: nitrous oxide. Jour. Indust. and Eng. Chem., 1911, iii, 8.

[7] Lunge: Ber. 14, 2188; Kemp: Chem. News, 71, 108 (1895).

[8] Landolt and Bornstein: Physikalisch-Chemische Tabellen, 1912, p. 150.

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molecules of nitrogen will be formed which will occupy 1.006 cc. So that we have

1.000 cc. N_2O	disappeared	1.006 cc. N_2	formed
1.006 cc. H_2	disappeared		
2.006 cc.	disappeared	1.006 cc.	formed

Therefore the contraction equals 1.000 cc., just equal to the volume of nitrous oxide.

If the nitrous oxide were P per cent pure, the contraction would equal the volume of nitrous oxide present in the sample taken with great exactness.

The only errrors lie in (1) the increase in "perfection" of nitrous oxide when mixed with an excess of hydrogen; (2) the "imperfection" of hydrogen; (3) the "imperfection" of nitrogen. These irregularities, however, are of the order of 0.05 per cent of the whole volume. And for the analysis of such small samples of nitrous oxide here described, which must be transferred, etc., this correction would be meaningless.[9]

Apparatus. For the analysis of nitrous oxide a carefully calibrated 10 cc. Haldane apparatus is used, modified as described by Krogh and Lindhard with a three-way hydrogen intake tap on the connecting piece leading to the combustion tube. The absorption pipette for carbon dioxide is filled with a concentrated solution of potash (sp. g. 1.55), instead of the usual 10 per cent solution. The alkaline pyrogallic solution must be very active, so that the oxygen will be quickly absorbed. The gas mixture should be carried into the potash and pyrogallic pipettes the same number of times in each analysis and check readings avoided. Under these conditions the error due to the solubility of nitrous oxide in these solutions is reduced to a point which is negligible when dealing with differences in percentages in dilute nitrous oxide mixtures.

The combustion tube is fitted with No. 35 platinum wire. For burning the nitrous oxide the wire should be bright red. A white heat is not necessary, but a dull red heat is not sufficient

[9] We are indebted to Dr. G. Shannon Forbes, Assistant Professor of Chemistry, Harvard University, for this exposition of the behavior of nitrous oxide in relation to the gas laws and to the method of analysis.

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to remove the last traces of nitrous oxide in an atmosphere consisting largely of hydrogen. In very dilute mixtures of nitrous oxide, when there is a large excess of hydrogen and only a comparatively small amount of nitrogen it is difficult to heat the wire to a bright red, as much more current is necessary under these conditions. For this reason we had to modify our resistance regulator so that a greater range of current strength was available.

Analysis of dilute mixtures. The analysis of mixtures containing a combined amount of oxygen and carbon dioxide not exceeding 22 per cent and 16 per cent of nitrous oxide can be made very readily and with great accuracy. Before proceeding with an analysis the apparatus should be tested for tightness by carrying through an air analysis, including the addition of hydrogen and heating of the combustion tube. This insures freeing the apparatus of carbon dioxide, oxygen, and nitrous oxide.

The sample is taken into the burette and measured. The carbon dioxide is absorbed by passing the sample back and forth into the potash tube eight times; the carbon dioxide contraction is then read. The oxygen is absorbed by passing the sample fifteen times into the pyrogallic, then washing the potash tube once, and again passing the sample into the pyrogallic eight times. The potash tube is once again washed and the sample is then finally passed into the pyrogallic eight times. The levels are adjusted and the oxygen contraction measured. About 2.5 cc. of hydrogen are drawn directly into the combustion tube and the platinum wire heated to remove all traces of oxygen. The hydrogen is then taken into the burette with the sample and the total volume measured. The mixture is combusted by passing the sample back and forth ten times over the heated platinum wire; the potash and pyrogallic tubes are washed once and the mixture passed into the combustion tube five times; the potash and pyrogallic tubes are again washed once each and the mixture combusted as before. The contraction is then read and this will be equivalent to the volume of nitrous oxide in the original sample. A check reading is made of this last volume, after again passing the gas into the potash and pyrogallic and combustion tubes.

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Very constant results are obtained when the analyses are carried out according to a definite technic and care used in adjusting the various levels. The level of the mercury in the combustion tube should be made with the three-way tap in connection with the burette and potash tube, otherwise serious error may be introduced by the production of pressure differences when obtaining the mercury level. Furthermore the readings should not be made after a combustion until the combustion tube is cool. A complete analysis usually takes about twenty minutes.

The exactness of the method is shown by the following results obtained by analysis of two samples of the same mixture:

	Sample I per cent	*Sample II* per cent
CO_2	0.10	0.10
O_2	17.79	17.77
N_2O	15.21	15.19

Analysis of concentrated mixtures. For the analysis of concentrated mixtures of nitrous oxide the following modification is necessary. Before beginning an analysis the apparatus is filled with about 7.1 cc. of hydrogen and all traces of carbon dioxide, oxygen, and nitrous oxide removed by passing the gas back and forth into the various tubes and heating the platinum wire. The levels are carefully adjusted; the reading of the hydrogen volume (with more or less nitrogen) obtained; and then the hydrogen is carefully stored in the combustion tube. About 2.5 cc. of the nitrous oxide is drawn into the burette and the hydrogen is then brought back from the combustion tube into the burette. The levels are adjusted and the total volume read. The analysis is then carried through as described above.

If the nitrous oxide sample contains only very small amounts of carbon dioxide or oxygen, it is not fair to assume that the entire contraction observed on passing into the potash and pyrogallic pipettes is due to the presence of these gases because slight traces of nitrous oxide are absorbed by the solutions. For instance, in the following consecutive analyses of samples from the same tank of nitrous oxide, the first sample was analyzed as above; the second sample was passed through alkaline pyrogallic

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before entering the burette and combusted directly without going into the potash or pyrogallic pipettes; the other six samples were combusted directly without ever being brought into contact with the potash or pyrogallic.

Sample I Entering potash and pyrogallic tubes, then combusting		*Sample II* Bubbling first through pyrogallic and then combusting directly		*Samples III to VIII* Combusting directly without coming into contact with either potash or pyrogallic	
	per cent		*per cent*		*per cent*
CO_2	0.21			N_2O	98.8
				N_2O	99.1
				N_2O	97.6
O_2	0.57			N_2O	97.9
				N_2O	98.5
N_2O	97.28			N_2O	98.4
Total	98.1	N_2O	98.6	N_2O	98.3

As the total contraction of samples I and II fall within the variations shown by samples III to VIII it is evident that the nitrous oxide contains no appreciable trace of oxygen; for by the combustion method with hydrogen, if oxygen were present, its contraction volume would be three times its actual volume and the above agreement would be impossible if there were any appreciable amount of oxygen present in the samples. The method does not admit of the determination of less than 0.1 per cent of oxygen.

The presence of an appreciable amount of carbon dioxide is ruled out by passing the gas through baryta water.

Therefore we feel justified in concluding that the tank from which the above samples of nitrous oxide were obtained contained 98.3 per cent nitrous oxide and 1.7 per cent nitrogen (argon or other noncombustible gases) with no appreciable amount of carbon dioxide or oxygen.

The method is particularly serviceable for analyzing commercial nitrous oxide used for anaesthetic purposes when the proportions of nitrous oxide, oxygen, carbon dioxide, and nitrogen are desired; it is not suitable for detecting traces of these or other contaminating gases.

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SUMMARY

The details are given of analysis of nitrous oxide by the method of combusting with hydrogen with the use of a modified Haldane apparatus.

The accidental errors of the method for dilute mixtures of nitrous oxide are slightly greater than are those for the analysis of air in a Haldane apparatus on account of the necessity of adding hydrogen and using the combustion tube. Furthermore there is a slight constant error caused by the absorption of nitrous oxide by the potash and pyrogallic solutions. As this error is practically the same in consecutive samples, if an identical analytical routine is used it can be neglected when comparative percentages are desired.

For the analysis of concentrated mixtures of nitrous oxide the Haldane apparatus does not admit of the use of a sample exceeding 2.5 cc. In consequence the errors of the apparatus as well as those of absorption are much magnified. As the errors of the apparatus are accidental the average of several analyses will minimize their importance. Duplicate analyses with and without the use of potash and pyrogallic, as well as analysis of samples collected over pyrogallic will allow the determination of the absorption error. A series of eight such analyses from the same commercial nitrous oxide tank gave an average of 98.4 per cent of nitrous oxide, the balance being nitrogen (argon or other non-combustible gases); the extremes were 97.6 per cent and 99.1 per cent. By taking the average of several analyses the purity of a sample of nitrous oxide can be determined by this method with an accuracy sufficient for most physiological purposes.

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THE EFFECT OF WORK ON THE PERCENTAGE OF HAEMOGLOBIN AND NUMBER OF RED CORPUSCLES IN THE BLOOD

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Tornow[1] found in experiments on soldiers before and after long marches that the red corpuscles in the capillary blood of the ear increased about 9 per cent and the white about 43 per cent. He considered that this increase in the red cells corresponded roughly to the increased density of the blood as a result of the sweat caused by the muscular work.

Some years later (1908) Hasselbalch and Heyerdahl[2] in a large series of experiments dealing with the effect of work on the increase in the number of leucocytes performed two experiments in which they determined the effect on the number of red cells. In both of these experiments they found an increase in the red corpuscles; in one ("Exp. 59"), there was a 17 per cent, and in the other ("Exp. 60"), a 13 per cent increase. The details of these two experiments are shown in Table I.

In neither of these experiments is there a definite reaction after the first run, while after the second run there is in both experiments a very distinct rise in the number of red blood corpuscles. This can be explained on the ground that there was no distinct change in the relative number of red cells until sufficient time had elapsed for an appreciable amount of sweating to have occurred.

[1] Tornow: Quoted by Hasselbalch and Heyerdahl, loc. cit., p. 291.

[2] Hasselbalch and Heyerdahl: Uber einige physische Ursachen zu Schwankungen der Menge von Blutkorperchern. Skand. Arch. f. Physiol., 1907–1908, xx, 289–329.

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In view of these findings it is evident, at least in certain subjects, that work causes a change in the oxygen carrying capacity of the blood. Therefore, in order to calculate properly the percentage saturation of the haemoglobin in the venous blood in a series of experiments being done in this laboratory on the determination of the blood flow at rest and at work, it was necessary for us to verify the above findings and to determine the order of the variation in the subject of the blood flow experiments.[3]

TABLE I

EXPER. NO.	TIME	POSITION	PULSE	RED CORPUSCLES	INCREASE
				per cu. mm.	*per cent*
59	10.10	Lying down	66	5,281,000	
59	10.17	Lying down after a sharp run		5,471,000	
59	11.23	Lying down	72	5,611,000	
59	11.34	Lying down after a second sharp run	128	6,354,000	17
60	9.30	Lying down	68	5,864,000	
60	9.40	Standing after a sharp run	130	5,717.000	
60	10.30	Lying down	80	5,554.000	
60	10.40	Standing after a second sharp run	138	6,487,000	13

For the determination of the haemoglobin use was made of the Haldane-Gowers haemoglobinometer and the colorimetric readings were controlled by Mr. H. F. Aitken, artist to Dr. Cushing.

The number of red corpuscles was determined in the usual way by counting and taking the average of four fields.

Before taking the samples of blood for determining the normal or resting value, the subject sat down for about five or ten minutes and care was taken that this period was preceded by only light laboratory work.

[3] Boothby: A determination of the circulation rate in man at rest and at work. The regulation of the circulation. This Journal, 1915, xxxvii.

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For determining the effect of work, the subject mounted a stationary bicycle and peddled for one-half hour before the blood sample was taken. During the latter part of the work period the subject exercised his arm and hand muscles in order that the blood sample would be obtained from an actively circulating blood current. In some of the experiments the respiratory exchange was determined just before taking the blood sample, in order to find the oxygen consumption per minute and thus have a quantitative expression of the amount of work done.

The results of the experiments are given in Table III at the end of the paper. In Table II are given the averages for each individual.

TABLE II

SUBJECT	HAEMOGLOBIN			RED CORPUSCLES		
	Rest	Work	Increase	Rest	Work	Increase
			per cent			*per cent*
W. M. B.	120	121	0	6,320,000	6,404,000	0
F. B. B.	101	108	7	6,283,000	7,333,000	17
H. F. A.	112	124	11	5,164,000	5,920,000	15
W. B. Y.	112	123	10			
J. W. W.	117	130	11			
F. G. W.	123	135	11	5,784,000	7,224,000	25

In W. M. B. no appreciable change occurs in the values found at rest and after work for either the percentage of haemoglobin or the number of red cells. In the other subjects there is a distinct rise in the haemoglobin percentage in all but one of the work experiments on F. B. B.; the determination of the red blood corpuscles followed in a general way the increase in the haemoglobin percentage, although the percentage increase appeared distinctly larger. The subject W. M. B., in whom no demonstrable change occurred either in the haemoglobin percentage or in the number of red corpuscles after work, perspires very little even in extremely warm weather. Under the conditions of the experiments, as here carried out, no appreciable amount of perspiration was formed and the skin remained dry during the entire experiment. Likewise in the experiment on F. B. B. on

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September first in which no change occurred in the haemoglobin percentage, the subject had not started to perspire when the blood sample was taken; fifteen minutes later the number of red blood cells showed a distinct increase and by this time the subject was perspiring very freely. In all the other experiments the subjects always perspired freely.

Our experiments which show an increase in the haemoglobin percentage and the number of red blood corpuscles, whenever the work performed caused the subject to perspire, substantiate the theory suggested by Tornow that the increase in the number of the red blood corpuscles, which he found in soldiers after long marches, was due to an increase in the density of the blood as a result of the sweat thereby produced.

On account of the rise in the haemoglobin percentage the oxygen carrying capacity per unit volume of blood is proportionately increased. It is another interesting example of the intercompensatory mechanism of the human body; it is evident that, in the main, the object of perspiration is to keep the body temperature down under conditions of work, yet its formation and elimination, by increasing the proportion of haemoglobin, increases the carrying capacity of a unit volume of blood thereby throwing less strain on the circulatory system.

It is obviously necessary, therefore, to determine under the actual experimental conditions the haemoglobin present in work experiments the object of which is to determine such factors as the oxygen carrying capacity of the blood, the coefficient of utilization of the oxygen per unit of blood, or in accumulating evidence for or against the secretory theory of oxygen, or other problems of like nature.

SUMMARY

Experimental data are given showing that the percentage of haemoglobin and the number of red blood corpuscles, and therefore the oxygen carrying capacity of a unit volume of blood, are increased under conditions of work, causing an appreciable amount of perspiration. If no perspiration occurs there is no such increase.

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TABLE III

DATE	SUBJECT	REST			WORK			INCREASE	
		O_2 Consumption	Haemoglobin	Red Corpuscles	O_2 Consumption	Haemoglobin	Red Corpuscles	Haemoglobin	Red Corpuscles
		cc. per min.	*per cent*	*per cu. mm.*	*cc. per min.*	*per cent*	*per cu. mm.*	*per cent*	*per cent*
Aug. 6	W. M. B.	(185)	120		1165	120			
Aug. 10	W. M. B.	(185)	120		924	120			
Aug. 13	W. M. B.	(185)	118	6,440,000					
Sept. 10	W. M. B.	(185)	123	6,200,000	(1000)	124	6,404,000		
			120	6,320,000		121	6,404,000	0	0
Aug. 7	F. B. B.	(225)	99		934	100			
Aug. 12	F. B. B.	(225)	99						
Aug. 14	F. B. B.	(225)	99		2289	111	7,832,000		
Aug. 15	F. B. B.	(225)	99		1946	100[1]	8,416,000		
Aug. 15	F. B. B.	(225)	99						
Aug. 15	F. B. B.	(225)				107[2]			
Aug. 17	F. B. B.	(225)	99		1934	112	7,208,000		
Aug. 18	F. B. B.	(225)	102		2164	111	6,080,000		
Aug. 21	F. B. B.	(225)	102						
Aug. 21	F. B. B.	(225)	102	6,048,000					
Aug. 22	F. B. B.	(225)		6,530,000					
Sept. 1	F. B. B.	(225)	101	6,272,000		101[3]			
Sept. 1	F. B. B.	(225)					7,280,000[4]		
Sept. 4	F. B. B.	(225)	102			110	7,184,000		
Jan. 15	F. B. B.	(225)	104			110			
			101	6,283,000		108	7,333,000	7	17
Sept. 11	H. F. A.		112	5,164,000	(1500)	124	5,920,000	11	15
Jan. 28	W. B. Y.		112			123		10	
Jan. 28	J. W. W.		117			130		11	
Jan. 28	F. G. W.		123	5,784,000		135	7,224,000	11	25

[1] Haemoglobin turned slightly brown (not averaged).
[2] Sample taken 50 minutes after work stopped.
[3] Subject not perspiring when blood sample was taken.
[4] Sample taken 15 minutes after haemoglobin sample.

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A DETERMINATION OF THE CIRCULATION RATE IN MAN AT REST AND AT WORK

THE REGULATION OF THE CIRCULATION

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CLINIC OF PROFESSOR CUSHING

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Bornstein[1] in 1910 introduced the principle of measuring the volume of blood per minute passing through the lungs of man by calculating, from the tension difference existing between the gas (nitrogen) in the alveolar air and in the blood, the quantity absorbed by the blood from the lungs in a known time. The next year Markoff, Muller, and Zuntz[2] improved the method by using nitrous oxide instead of nitrogen.

Krogh and Lindhard[3] in 1912 nearly coincidently with Markoff, Muller, and Zuntz employed nitrous oxide and developed the method to a high degree of accuracy. Krogh[4] has recently shown, however, that an error of the order of about 6 per cent exists in all the blood flow experiments given in the above paper, as well as in recent papers by Lindhard,[5] because in the calcula-

[1] Bornstein: Eine Methode zur vergleichenden Messung des Herzschlagvolumens beim Menschen. Pflugers Archiv 1910, cxxxii, pp. 307–318.

[2] Markoff, Muller, and Zuntz: Eine Stickoxydul-Methode zur Bestimmung der umlaufenden Blutmenge in lebenden Korper. Ztschft. f. Balneologie, 1912, iv, 14–15.

[3] Krogh and Lindhard: Measurement of the blood flow through the lungs of man. Skand. Archives f. Physiol., 1912, xxvii, pp. 100–125.

[4] Krogh: Funktionsuntersuchungen an den Lungen des Menschen mittelst gasanlytischer Methoden; Abderhalden: Handbuch der Biochem. Arbeitsmethoden. 1915, Urban und Schwarzenberg. pp. 550–558.

[5] Lindhard: Concerning the influence of ultraviolet light on the circulation in man. Skand. Archives f. Physiol., 1913, xxx, pp. 73–96; Lindhard: Effect of posture on the output of the heart. Skand. Archiv. f. Physiol., 1913, xxx, 395–408.

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tions the coefficient of absorption of nitrous oxide for blood has been taken as 0.43, as determined by Siebeck in 1909 for ox blood at 37°. Lindhard and Krogh[6] have recently determined the coefficient of absorption of nitrous oxide for human blood at 37° and found it to be 0.405 ± 0.005.

The method used by us in the present study is essentially that given by Krogh in his most recent paper.[7] However, as a few changes in the routine of experimentation have been made by us we present our technic in full.

EXPERIMENTAL DETAILS

Unit of work. The object of our experiments is to show the volume per minute of the circulation between a condition of complete rest in bed to one of severe muscular work. It was necessary to have a method of expressing the amount of work performed by the subject under all the conditions of the experiments. A mechanical unit, such as kilogram-meters per minute, gives a rough comparative idea of the amount of work performed by the subject while pedaling an ergometer, such as that described by Krogh.[8] This unit, however, cannot be used to compare the amount of work a subject's muscles would be called upon to perform when sitting at rest on a bicycle, in a chair, or lying in bed; further, it does not take into consideration variations produced in efficiency by a proper position on the seat or the energy expended by the arm muscles exerting a counter pull on the handle bars of the bicycle. We have, therefore, adopted the physiological unit of the number of cubic centimeters of oxygen consumed per minute.

For the study of the circulation rate, the unit of oxygen consumption per minute is particularly well adapted, for it is one of the chief functions of the blood to transport the gases between the lungs and tissues. In fact the transportation of the other metabolic substances of the blood current, though equally essen-

[6] Krogh: See reference 4, p. 550.

[7] Krogh: See reference 4.

[8] Krogh: A bicycle ergometer and respiration apparatus for the experimental study of muscular work. Skand. Archiv. f. Physiol., 1913, xxx, 375–394.

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tial to the organism, are probably comparatively small from the point of view of bulk per unit volume of blood, though of great importance from the point of view of their effect on the gaseous carrying capacity of a unit volume of blood.

Body equilibrium. The gaseous equilibrium of the body for the condition of the experiment is established by having the subject maintain such a condition of rest or of work for at least one-half hour before any experimental observations are made.

Rest. For the experiments at complete rest, the subject lay on his back in bed without moving throughout the entire preliminary and experimental periods. The various mouthpieces were supported on a bar, and the necessary manipulations of changing from one to another were performed for him. The experiments were usually done about the middle of the forenoon after an ordinary breakfast. It was not considered necessary to insist on a basic, fasting condition, as the study was not the determination of the lowest possible circulation rate but the volume per minute of blood necessary to transport a unit volume of oxygen. In fact a slight variation from day to day in the oxygen consumption would be advantageous as it might reveal corresponding changes in the blood flow. In addition to the experiments at complete rest others were done with the subject seated in a straight-backed chair; otherwise no change in the routine was made.

Work. For the work experiments a stationary bicycle with the rear wheel heavily balanced by a lead pipe tire and suitably mounted was used. The degree of work performed by the subject was regulated by changing the resistance of a friction brake, or by varying the speed of pedaling. During a single experiment, including the preliminary period for establishing equilibrium, the brake resistance and the rate of pedaling were maintained constant, the latter was accomplished by pedaling in time to the ticking of a metronome.

Respiratory exchange. The oxygen consumption per minute was determined by making a complete respiratory exchange

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experiment after equilibrium was established and just before the circulation experiment proper. The subject breathed through a set of Douglas valves into a 30-litre collecting spirometer. The surfaces of the valve-discs were covered with a layer of thick machine oil; in addition, a small loop of wire was passed around the cross wires over the valve-disc and the free end rested on the disc increasing its weight slightly. These changes reduced the leakage below an appreciable point without noticeably increasing the resistance to the passage of air.

The spirometer was carefully calibrated and the air volumes could be determined within 50 cc. an error of 0.2 per cent for 25 litres. The air samples were analyzed in a regular 10 cc. Haldane[9] gas analysis apparatus, the error of which for routine work does not exceed 0.02 per cent. The duration of the respiration experiment was obtained by a stop-watch and determined to a fifth of a second, which would give an error of 0.05 per cent for the rest and 0.3 per cent for the work experiments. The tap was turned at the bottom of expiration both at the beginning and at the end of an experiment. The carbon dioxide elimination and the oxygen consumption per minute were calculated in the usual way, allowing for the change in volume of the expired air.[10] To avoid the necessity of making a correction for the dead space of the spirometer it was filled with expired air and then emptied just before beginning the experiment.

Temperatures. The temperature of the gas in the collecting and recording spirometers was taken as that of the water bath at the time of measurement of the gas volume and read to 0.1°C. and the gas was assumed to be fully saturated with water vapor. The temperature of the gas in the lungs and air-passages was taken as 37°, and likewise assumed to be fully saturated with water vapor.

Barometer. The atmospheric pressure was determined by a mercury barometer of the U. S. Weather Bureau type and read by means of a vernier to 0.1 mm. The observed reading was

[9] Haldane: Methods of air analysis. 1912, Charles Griffin & Co., London.

[10] Haldane: Methods of air analysis (p. 56). 1912, Charles Griffin & Co., London.

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always corrected to 0°C. The barometer was read at the time of measuring of the gas volumes.

Pulse rate. To obtain the pulse rate both at the time of the respiration and circulation experiments, an ordinary 6-inch clinical blood pressure cuff was placed on the subject's arm. The cuff was inflated to a point just above the diastolic blood pressure and was connected to a tambour or mercury manometer which wrote on the kymograph under the tracing made by the recording spirometer. The cuff could be worn with comfort for periods as long as fifteen minutes.

Recording spirometer. A carefully calibrated 6-litre Krogh recording spirometer was used to contain the nitrous oxide mixture. From the spirometer curve it was possible to read the volumes with an average error not exceeding 10 cc. and as the differences in volume were more than 1000 cc. the error was less than 1.0 per cent. To insure the thorough mixing of the gases a small fan on the floor of the spirometer was kept running throughout an experiment by means of a small electric motor.

The preliminary gas mixture was made by introducing into the spirometer 4.5 L. of air, 0.5 L. of oxygen, and 1.0 L. of nitrous oxide. After thorough mixing the mouth tap was opened so as to fill this and the connecting tube with the mixture.

A piece of garden hose of 20 mm. inside diameter and about 20 cm. long led from the spirometer to a three-way brass valve to which was attached a rubber mouthpiece with a wide flange that fitted in between the lips, cheeks, and teeth. By means of this valve the subject could be connected either with the spirometer, or the outside air, or the valve could be entirely closed. To the spirometer side of the valve was connected a flexible lead tube of 0.6 mm. bore by means of which alveolar air samples could be withdrawn into collecting tubes.

In the circulation experiments the time is written on the drum in seconds and can be read with an error not exceeding 0.1 seconds which would give for an experiment of 20 seconds an error of 0.5 per cent.

Analysis of nitrous oxide. The analysis of the dilute nitrous oxide mixture is accurate in routine work to within 0.05 per

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cent. If exceptional care is taken with an absolutely clean burette the percentages can be determined to within an error of 0.03 per cent. As the studies here presented are based on the accuracy of the nitrous oxide analyses we felt obliged, as a preliminary step, to satisfy ourselves of the fundamental accuracy of the analytical technic and the results of our studies are presented in a separate article.[11]

Residual air. As the volume of the subject's residual air is necessary in making the calculation of the total volume of the nitrous oxide mixture in the lungs, this must be determined by preliminary experiments. This we did by the usual hydrogen method the details of which need not be given here. Unlike Krogh,[12] we could find no difference in the volume of the residual air whether at rest or at work, therefore we have used the same volume in the calculation of all our experiments. We are planning to elaborate on this point in a separate article.

The circulation experiment proper. The mouthpiece of the recording spirometer is adjusted in the subject's mouth so that it will be air-tight, and the nose is closed with a clip. The subject breathes through this valve to the room air for two or three minutes being careful to maintain the same rate of pedaling in the work experiments or to keep absolutely quiet in the rest experiments. On signal he gives the greatest possible expiration at the end of which the observer turns the tap so that the following inspiration consists of the nitrous oxide mixture in the spirometer. When the subject has inspired an easy maximum, the observer closes the tap so that the subject holds his breath for five to eight seconds, in order that the lung tissues and the blood already in the lungs may reach an equilibrium with the tension of the nitrous oxide in the alveolar air. Then the observer opens the tap to the spirometer and the subject gives a sharp expiration of such a volume that he expires as near as possible to his "Mittelage." The observer closes the tap and the subject holds his breath for fifteen to twenty seconds.

[11] Boothby and Sandiford: The analysis of nitrous oxide for physiological work. This Journal, 1915, xxxvii.

[12] Krogh: See reference 4, pp. 536–539.

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As soon as the tap is closed the observer withdraws a sample of the expired air (Sample No. 1). Then on signal the observer again opens the tap to the spirometer and the subject expires very deeply and quickly; the tap is then turned to the room air, and a sample of the second expiration is taken (Sample No. 2).

It seems reasonable to assume *under constant conditions of metabolism*, that the gaseous content of the venous blood as it enters the lungs does not appreciably vary from moment to moment. However, during an experiment in which the breath is held marked changes would naturally follow if any part of the total blood volume could during this time effect a complete circuit and reenter the lungs a second time. The probability of the presence of recirculating blood would depend on the relationship between the total blood volume and the rapidity of the circulation rate. The blood volume of the subject of these experiments has never been determined but judging from the figures given by Douglas,[13] it cannot be far from 3.8 L., allowing for the difference in weight. As the duration of the experiments at rest does not exceed twenty-five to thirty seconds and at work fifteen to eighteen seconds, and as the circulation rate under corresponding conditions is 3.4 and 9.3 litres per minute respectively, it is extremely unlikely that any blood would have time to return to the lungs except that of the coronary circulation. This latter would be of such a relatively small amount that no serious error is probably introduced by neglecting it.

The abnormal condition of the subject while holding his breath during the circulation experiment affects the blood flow markedly by altering the intrapulmonary air pressure. If this air pressure is high, less blood will tend to flow into the thoracic cage than if the air pressure is low. The amount of this variation in intrapulmonary pressure can in large part be obviated by making the expiration after the preliminary period of such a volume that the chest walls are in a position of elastic equilibrium;

[13] Douglas: The determination of the total oxygen capacity and blood volume at different altitudes by the carbon monoxide method. Jour. Physiol., 1910, xl, 6, 471–478.

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this position corresponds to the bottom of a normal expiration. Furthermore, the subject must not hold his thoracic muscles rigid during the experimental period because the volume of air in the lungs is decreasing; in consequence, the chest wall must

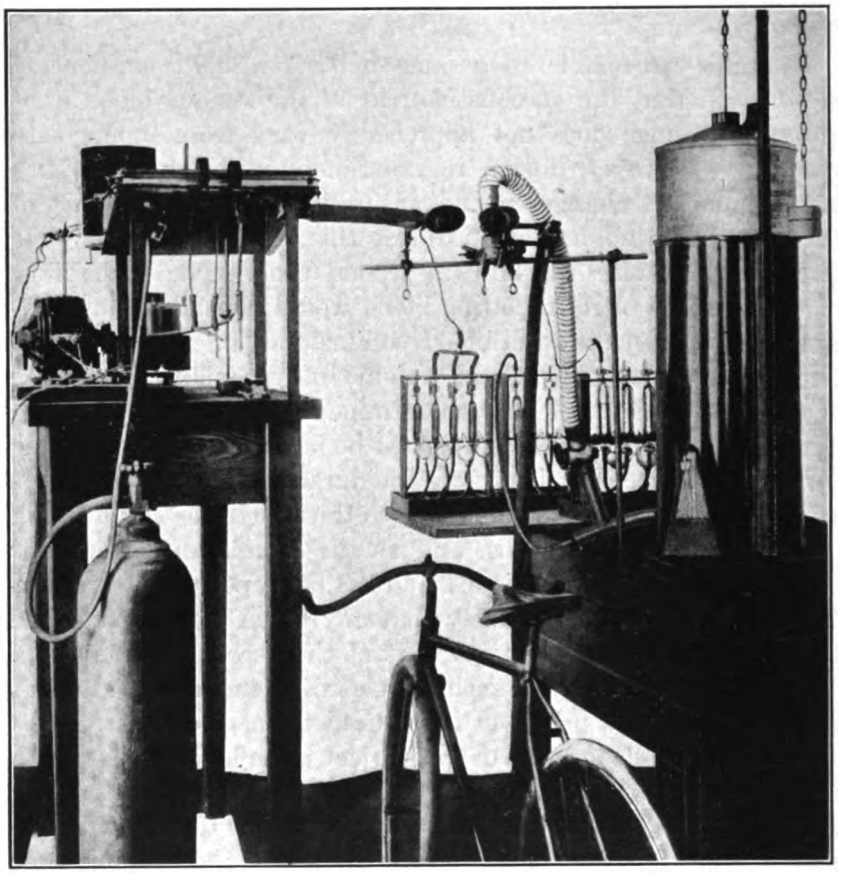


Fig. I. Arrangement of the apparatus for respiration and circulation experiments at work.

be allowed to contract down proportionately, otherwise a negative intrapulmonary pressure would be developed, thereby sucking into the lungs an excess amount of blood.

In practice it is impossible to expire to the exact position of

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elastic equilibrium therefore more or less blood may flow into the lungs on account of the disturbed pressure conditions inside the thorax than would normally flow under the metabolic conditions of the experiment. From the circulation experiment proper it is possible to determine the oxygen absorbed by the blood; by means of the preliminary respiration experiment under identical metabolic conditions the normal oxygen consumption for these conditions is known. Therefore as the oxygen content of the venous blood during an experimental period is constant, the difference in oxygen consumption will be proportional to the difference in the volume of the blood entering the lungs. In consequence the observed minute volume can be corrected by dividing it by the oxygen absorption found in the circulation experiment and then multiplying the result by the oxygen absorption determined in the preliminary respiration experiment (see protocol of circulation experiment).

PROTOCOL OF EXPERIMENT

Experiment No. 7, May 9, 1914; Subject, W. M. B., resting on bed.

Respiratory exchange experiment

Prelim. period.....30 min.	Bar.....755.3
Duration of exp....6.3 min.	Corr. Temp..... 2.8
Temp. spir.....22.5°	Corr. Bar.....752.5
	Temp. bar 23.0°

Reading spir.—End..... 29.40 L.
Reading spir.—Start..... 0.38 L.
Total volume..... 29.02 L. at 752.5 and 22.5° sat.
or..... 25.82 L. at 760 and 0° dry.

Analysis of air samples from the spirometer, in duplicate

	Sample 1	*Sample 2*
Sample	9.407 9.407 9.083	9.499 9.499 9.173
CO_2	9.083 = 0.324 = 3.44 per cent	9.173 = 0.326 = 3.43 per cent
O_2	7.492 7.492 = 1.591 = 16.91 per cent	7.565 7.565 = 1.608 = 16.93 per cent

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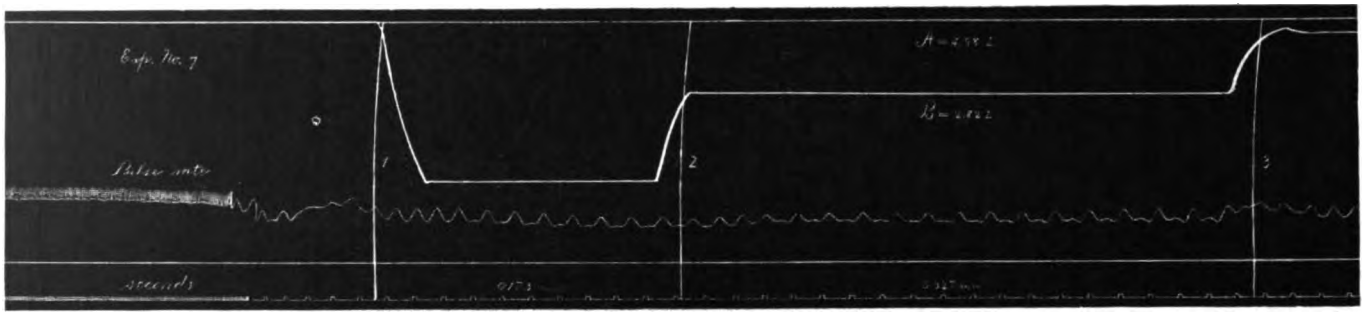


Fig. II. Spirometer and pulse tracing of experiment No. 7, May 9, 1914. See protocol.

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CO_2 = 3.44 per cent
O_2 = 16.92 per cent
N_2 = 79.64 per cent

$$20.93 \times \frac{79.64}{79.04} = 21.09 \text{ per cent}$$

CO_2 eliminated.

$3.44 - 0.03 = 3.41;$

$\frac{3.41}{100} \times 25.82 = 880$ cc. for 6.3 min.

or 140 cc. per min.

O_2 Absorbed.

$21.09 - 16.92 = 4.17;$

$\frac{4.17}{100} \times 25.82 = 1077$ cc. for 6.3 min.

or 171 cc. per min.

Resp. quo. $= \frac{140}{171} = 0.819$

Ventilation per minute $= \frac{25.82}{6.3} = 4.10$ L at 760 and 0° dry

or 5.01 L at 760 and 37° sat.

Circulation experiment proper

Introd. period..10.4 secs. = 0.173 min.
Exper. period..19.6 secs. = 0.327 min.
Temp. spir.....21.8°

Bar.....755.3
Corr. Temp.....2.8
Corr. Bar.....752.5
Temp. bar. 23.0°

Analysis of samples

	Sample 1			*Sample 2*		
	9.393			9.393		
Sample....	9.393			9.393		
Co_2.....	8.983	= 0.410 =	4.37 per cent	8.833	= 0.560 =	5.96 per cent
O_2.....	7.619	= 1.364 =	14.52 per cent	7.693	= 1.140 =	12.14 per cent
Adding H_2.	9.409			9.367		
	9.409			9.367		
	8.183			8.352		
N_2O.....	8.183	= 1.226 =	13.05 per cent	8.352	= 1.015 =	10.81 per cent
N_2.....			68.06 per cent			71.09 per cent

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Vol. air in spir. at end of exp..... 4.08 L (see Fig. II)
Vol. air in spir. during exp..... 2.82 L (see Fig. II)
Vol. air in lungs during exp..... 1.26 L
Vol. alv. resid. air..... 1.10 L
Total vol. in lungs during exp..... 2.36 L
Corrections:
Sample 1 = 25 cc.
6×10.4 = 62 cc.
87 cc. = 0.09 L
Vol. air in lungs at start of exp.....2.27 L
Vol. air in lungs at end of exp... $2.27 \times \frac{68.06}{71.09} = 2.17$ L.

O_2 Absorbed

$$2.27 \times \frac{14.52}{100} = 0.3296 \text{ L}$$
$$2.17 \times \frac{12.14}{100} = 0.2634 \text{ L}$$
0.0662 L at 752.5 and 21.8° sat.
or 0.0591 L at 760. and 0° dry.
$$\frac{0.0591}{0.327} = 181 \text{ cc. per min.}$$

N_2O Absorbed.

$$2.27 \times \frac{13.05}{100} = 0.2962 \text{ L}$$
$$2.17 \times \frac{10.81}{100} = 0.2346 \text{ L}$$
0.0616 L at 752.5 and 21.8° sat.
or 0.0550 L at 760. and 0° dry.
$$\frac{13.05 + 10.81}{2} = 11.93 \text{ per cent}$$
$$11.93 \times \frac{753-47}{760} = 11.93 \times \frac{706}{760} = 11.08 \text{ per cent}$$

Blood flow.

$$\frac{0.0550}{0.405 \times 0.1108 \times 0.327} = 3.75 \text{ L}$$

Blood flow corrected to normal exchange.

$$3.75 \times \frac{171}{181} = 3.54 \text{ L}$$

THE CALCULATION OF AN EXPERIMENT

The various steps in the calculation of an experiment have been given above in the protocol, most of which are self-evident. Several points in the calculation of the blood flow need, however, further elucidation.

The nitrogen percentage in Sample 2 has risen during the experiment and is due, as Krogh and Lindhard point out, to a

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slight liberation of nitrogen from the blood as well as to the contraction in volume of the air in the lungs because of the absorption of oxygen and nitrous oxide that is only partly offset by the elimination of carbon dioxide. The amount of nitrogen liberated from the blood is so small that it can be neglected, as Krogh and Lindhard have shown.

The volume of air from the spirometer curve is of course equal to the difference between *A* and *B* (see Fig. II). From other experiments we know the subject's alveolar residual air to be 1000 cc. (at 760 and 0° dry), and have corrected this for an average experimental temperature of 22.0°. Where there are great variations in experimental temperature from 22.0°, we have recalculated the residual air for that temperature.

The correction for the change in volume of the air in the lungs during the introductory period is calculated from that found in the experimental period. The volume of the air in the lungs at the end of the experiment is calculated from the percentage of nitrogen in the two samples:

$$2.27 \times \frac{68.06}{71.09} = 2.17 \text{ L.}$$

The contraction in volume of the air during the experimental period is therefore 2270 − 2170 = 100 cc. in 19.6 seconds. Or the change in volume per second is 5 cc. The contraction in volume of the air during the introductory period is taken to be the same as that during the experimental period. Krogh has taken it to be 6 cc. per second and we have found this a fair average for the rest experiments. In work, however, the volume change per second varies considerably and we have, therefore, calculated the value for each of the work experiments.

To determine the duration of the introductory and experimental periods lines are dropped from the respiration curve to the time curve. In doing this allowance must be made for the dead space as the instant at which the sample obtained actually left the lungs preceded the completion of the expiration by the time requisite for this volume of air to be expired. By other experiments we determined that the combined dead space of the subject and the valve was 125 cc.

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The quantity of N_2O in the lungs at the beginning of the experimental period is

$$2.27 \times \frac{13.05}{100} = 0.2962 \text{ L.}$$

At the end of the period it is

$$2.17 \times \frac{10.81}{100} = 0.2346 \text{ L.}$$

The quantity of N_2O absorbed is

$$0.2962 \text{ L.} - 0.2346 \text{ L.} = 0.0616 \text{ L. at } 752.5 \text{ and } 21.8° \text{ sat.}$$

or $$0.0550 \text{ L. at } 760 \text{ and } 0° \text{ dry.}$$

The mean percentage of N_2O in the lungs can be taken as

$$\frac{13.05 + 10.81}{2} = 11.93 \text{ per cent}$$

Or, expressed in per cents of an atmosphere (dry)

$$11.93 \times \frac{753 - 47}{760} = 11.93 \times \frac{706}{760} = 11.08 \text{ per cent}$$

The absorption coefficient of N_2O at 37° is 0.405 (Lindhard and Krogh.[14]) Therefore the volume of blood necessary to absorb 0.0550 L. of N_2O is

$$\frac{0.0550}{0.405 \times 0.1108} = 1.23 \text{ L.}$$

And this volume must have passed through the lungs during the 0.327 minutes of the experiment. Therefore the minute volume equals

$$\frac{1.23}{0.327} = 3.75 \text{ L.}$$

The oxygen absorbed during the circulation experiment proper is calculated in the same manner as that of the N_2O (see protocol).

From the spirometer experiment preceding the blood flow experiment the oxygen absorbed per minute was found to be 171 cc. The corrected blood flow is therefore

[14] Krogh: See reference 4, p. 550.

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$$3.75 \times \frac{171}{181} = 3.54 \text{ L.}$$

Instead of reducing the volume of air in the lungs, Krogh corrects the percentages in the second sample to what they would have been had the volume remained the same. The above analyses would have been corrected thus.

	Sample 1	Sample 2	Sample 2 Corr.	1–2 Corr.	$\frac{1+2}{2}$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
CO_2	4.37	5.96	5.71		
O_2	14.52	12.14	11.62	2.90	
N_2O	13.05	10.81	10.35	2.70	11.93
N_2	68.06	71.09			

The blood flow is then calculated as follows:

$$\frac{2.27 \times .0.0270}{0.405 \times 0.1193 \times 0.327} = 3.87 \text{ L.}$$

This blood flow must be corrected, however, since the volume of air in the lungs (2.27 L.) has not been reduced to standard conditions and the mean tension of nitrous oxide (11.93 per cent) should be expressed in percentage of an atmosphere. Krogh finds that these two corrections counterbalance each other almost exactly leaving only a slight correction,[15] dependent on the temperature, by which the above result must be multiplied. The above blood flow then becomes:

$$3.87 \times \frac{96}{100} = 3.72 \text{ L.}$$

According to the short method the entire calculation can be made as follows:

$$\frac{2.27 \times 0.0270 \times 0.96}{0.405 \times 0.1193 \times 0.327} = 3.72 \text{ L.}$$

[15] Krogh: See reference 4.

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The correction table as given by Krogh is as follows:

Temp.	*Correction*
14°	1.00
16°	0.99
18°	0.98
20°	0.97

From the above calculations it will be seen that this factor, though not absolutely correct, is sufficiently so for most purposes. For the ranges in temperature and pressure in our experiments the factor varies between $\frac{94}{100}$ and $\frac{99}{100}$. Krogh's table does not allow for variations in the barometer, so that we have preferred to calculate all our experiments by the longer method.

Krogh states that the exactness of the experimental method allows determinations of the blood flow to be made within an error of 10 per cent. We believe this to be a very fair estimate of the accuracy of the method.

The nitrous oxide method of determining the blood flow, as has been pointed out above, possesses what may be considered as constant and accidental errors. The chief constant errors are inherent in the assumption that the blood, while passing through the lungs, reaches a condition of tension equilibrium with the nitrous oxide in the alveoli and that during the course of an experiment no appreciable amount of blood recirculates and enters the lungs for a second time. These errors, if they exist, cannot at the present time be obviated. The accidental errors also on account of the technical difficulties of the experiment may be numerous. We have attempted to reduce the influence of the accidental errors by performing a reasonably extensive series of experiments. We realize, however, that a much larger number would be highly desirable and regret that, for the present, stress of work on other lines prevents us from doing more experiments. We cannot, therefore, insist on the absolute accuracy of our blood flow data, though we believe the comparative figures can be considered as being reasonably correct. The theoretical venous carbon dioxide and oxygen ten-

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sions, as calculated from our blood flow data, are obviously open to the same criticism. As a control, however, we have made a number of direct determinations of the venous carbon dioxide and oxygen tensions according to the method suggested by Christiansen, Douglas, and Haldane[16] and they agree with the calculated results here given in a very satisfactory manner. We hope shortly to complete a sufficient series for publication.

The essential data with the calculated results of all our experiments are given at the end of the paper in Tables II, III, and IV. These results are averaged into groups and presented in a summarized form in Table I.

TABLE I

O_2 CONSUMPTION PER MIN.	BLOOD FLOW PER MIN.	PULSE RATE PER MIN.	TOTAL VENTILATION AT 37° SAT. AT PREVAILING BAR. PRESS. PER MIN.
cc.	*L*		*L*
175	3.37	58	5.5
185	3.57	58	6.7
320	5.06	75	9.8
448	5.30	87	12.8
559	6.54	96	15.5
608	7.59	91	16.8
912	9.31	133	24.1

Weight subject, 48 kilos.

The averages of the calculated results from Table I are plotted in Figure III in which the ordinates represent the volume or amount of the various factors and the abcissa is the oxygen consumption per minute expressed in cubic centimeters and at standard temperature and pressure dry. In Figure IV these curves are repeated with the addition of several secondary curves. An explanation and discussion of the construction of the curves in this figure follow.

I. Blood flow. The averages for the blood flow determinations show a progressive though slightly irregular increase with the oxygen consumption. We have constructed a straight line

[16] Christiansen, Douglas, and Haldane: The absorption and dissociation of carbon dioxide by human blood. Jour. Physiol., 1914, xlviii, 4, 244–271.

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to represent the increase in blood flow, as such a line passes nearer to all the plotted points than would a simple curve. From considerations elaborated fully on page 413 it is highly

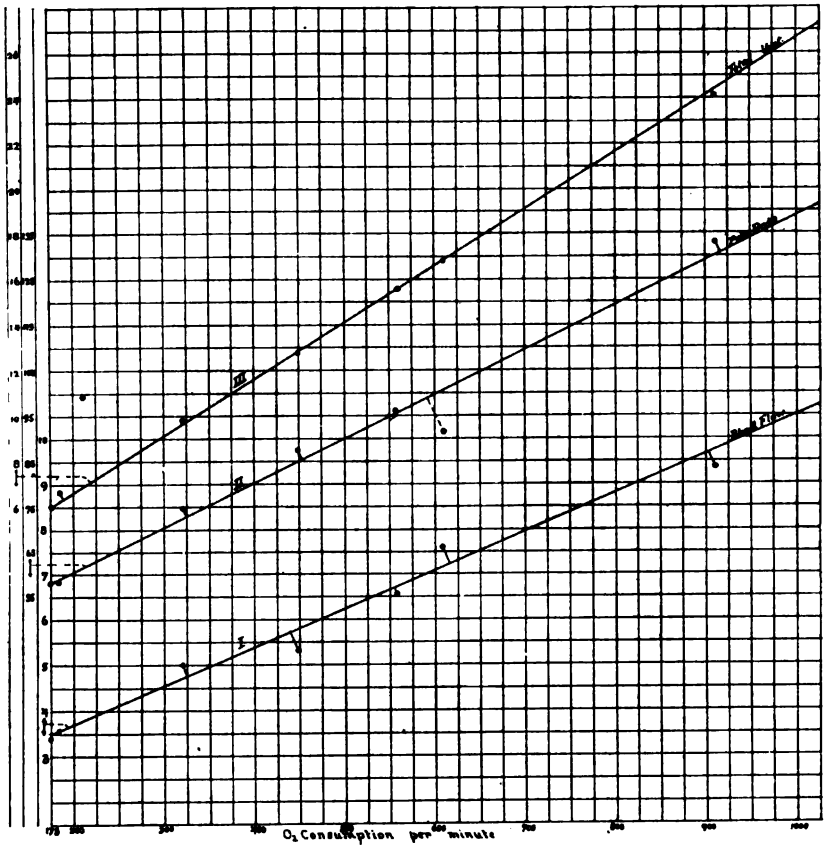


Fig. III. Curve I, blood flow. Curve II, pulse rate. Curve III, total ventilation. For description of curves see text. Ordinate represents litres per minute for blood flow; and total ventilation; and beats per minute for pulse rate. Abcissa is the oxygen consumption per minute in cubic centimeters.

probable that the blood flow follows some definite law of increase corresponding to its increased functions. The other two mechanical factors concerned with the transportation of the respiratory gases which are directly measurable are the total venti-

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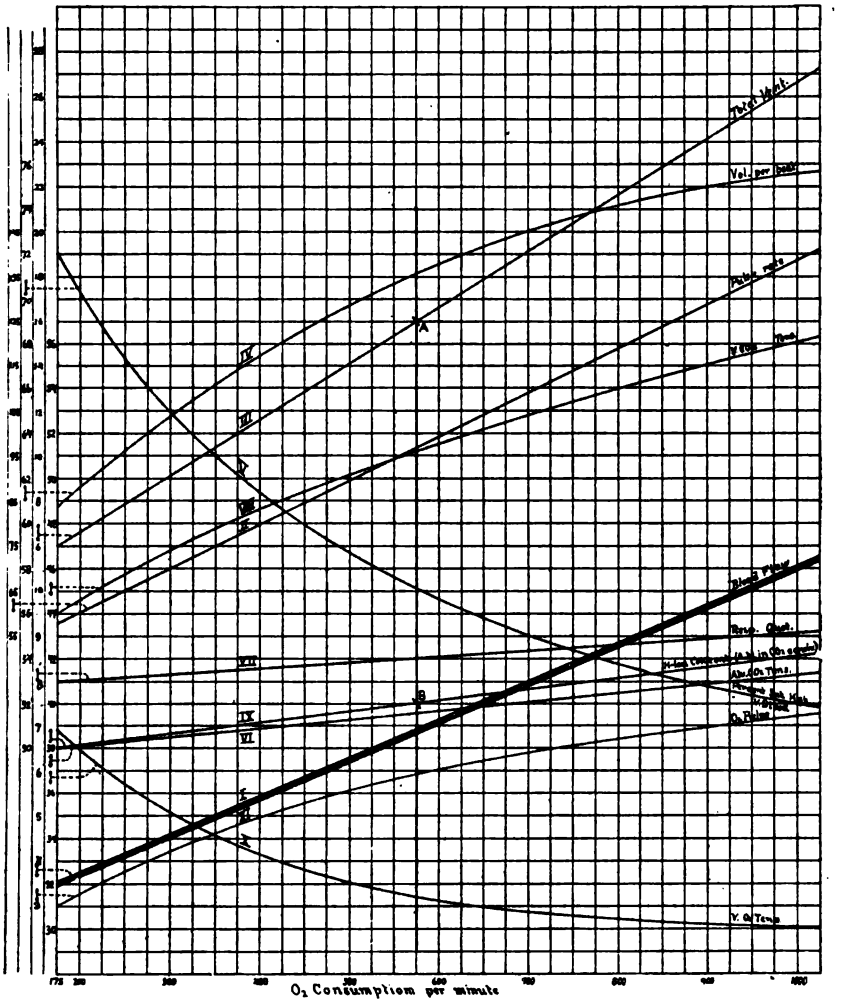


Fig. IV. Curves I, II, and III are the same as in the preceding figure, except that the plotted points representing the data are eliminated. The remaining curves are calculated from the three primary curves as described in text. Curve IV, volume per beat. Curve V, percentage saturation of the haemoglobin in the mixed venous blood. Curve VI, alveolar CO_2 tension. Curve VII, respiratory quotient. Curve VIII, tension of CO_2 in the venous blood, allowing for the influence of the percentage saturation of the haemoglobin with oxygen. Curve IX, hydrogen ion concentration of the arterial blood (for construction see text). Curve X, tension of oxygen in the venous blood allowing for the influence of the total acidity. Curve XI, oxygen pulse (Henderson).

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lation and the pulse rate. From Curves II and III, it is seen that these factors increase, on the average, according to a straight line; that they do so lends probability to the correctness of the assumption on which the blood flow curve is constructed and that the divergence of the plotted points from the line is not beyond the limits of error of the experimental method. The divergence of the various experimental points from the straight line is shown by the following:

Position of point cc.	*Divergence from curve I* per cent
175	0.6
185	0.3
320	7.0
448	8.6
559	2.8
608	6.2
912	3.9

The values as found by us for man are consistent with those obtained by Patterson and Starling[17] in their heart-lung preparations.

II. Pulse rate. The points representing the pulse rate fall within 3 per cent of a straight line with the exception of one corresponding to an oxygen consumption of 608 cc.; this point is 9 per cent off the line. It is evident, therefore, that in the main the pulse rate increases with the oxygen consumption, though in individual instances nervous or other influences may modify the rate somewhat, as can be seen in the tables.

III. Total ventilation. The various points representing total ventilation (37° saturated and existing barometer) likewise fall very close to a straight line with the exception of that corresponding to 185 cc. oxygen consumption. This may possibly be explained by the fact that the determination at 175 cc. was made with the subject lying down and that at 185 cc. was made while sitting in a chair. The change in position might have had an appreciable effect on the ventilation.

[17] Patterson and Starling: On the mechanical factors which determine the output of the ventricles. Jour. Physiol., 1914. xlviii, 5, 357–380.

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IV. Volume per beat. The curve representing the volume of blood pumped by the heart at each stroke was determined by dividing the volume per minute, as read from the blood flow curve, by the number of beats per minute, as shown by the pulse rate curve. This calculation was made for 175, 200, 300, 400, 500, 600, 700, 800, and 900 cc. of oxygen consumption. Likewise in the construction of the other secondary curves we have made the calculations directly from the primary curves and not from the actual experimental points. It is evident that the volume per beat increases at first quite rapidly and then very much more slowly.

In individual experiments the heart rate and the volume per beat vary inversely. The output per minute is, therefore, as Patterson and Starling[18] show, probably dependent on the amount of blood flowing into the heart.

V. Percentage saturation of the haemoglobin. The percentage saturation of the haemoglobin in the venous blood can be calculated as follows. In the subject of these experiments the haemoglobin per cent is 120, as determined by the Haldane-Gower's haemoglobinometer. Within the limits of the work and under the conditions existing in the blood flow experiments, there was no appreciable change in the haemoglobin percentage of the subject whether at rest or at work. This fact, however, must be experimentally determined, because individuals vary greatly, as shown by Boothby and Berry.[19] The amount of oxygen carried by a litre of fully saturated blood would then be $185 \times \frac{120}{100} = 222$; to which must be added 3.0 cc. in the plasma, making a total of 225 cc. However, Cook and Barcroft[20] have shown that the haemoglobin, as it leaves the lungs, is only 94 per cent saturated. Therefore the arterial blood con-

[18] Patterson and Starling: On the mechanical factors which determine the output of the ventricles. Jour. Physiol., 1914, xlviii, 5, 357–380.

[19] Boothby and Berry: Effects of work on the percentage of haemoglobin and number of red corpuscles in the blood. This Journal, 1915, xxxvii.

[20] Cook and Barcroft: Direct determination of percentage saturation of the arterial blood with oxygen in a normal person. Jour. Physiol., 1913, xlvii; Proc. Phys. Soc., xxxv.

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tains only 212 cc. of oxygen: $222 \times \frac{94}{100} = 212$ cc. The percentage saturation of haemoglobin in arterial blood during work has never been determined experimentally, so far as we know. We have been obliged, therefore, to use the same figure (212 cc.) for both rest and work, thus possibly introducing a slight error. For 300 cc. oxygen consumption per minute the oxygen per litre of blood is obtained by dividing by the blood flow (4.53 L.) $\frac{300}{4.53} = 66$ cc. If 66 cc. oxygen per litre is used then the venous blood contains $212 - 66 = 146$ cc. As the total oxygen capacity is 225 cc.[21] then the percentage saturation of the venous blood is $\frac{146}{225} = 64.9$ per cent. The form of the percentage saturation curve is remarkably interesting and it illustrates the economical interrelationship of the various factors concerned in the body metabolism.

VI. The alveolar carbon dioxide tension. The points representing the alveolar carbon dioxide tension were in a few instances obtained in the circulation experiments. They were mostly obtained, however, by an independent study. The method in brief was to take alveolar air samples by the Haldane-Priestly method before beginning work and again about twenty minutes after commencing to work, by which time equilibrium was assumed to be established. Although there were exceptions both above and below, we obtained an average rise of 2 mm. in the alveolar carbon dioxide tension above the resting value, when work was done requiring 700 cc. of oxygen consumption per minute. Krogh and Lindhard[22] have recently criticised the Haldane-Priestly samples under conditions of work as being likely to give too high a percentage for carbon dioxide and too low for oxygen. The error is due, according to them, to an extra

[21] In this calculation we have assumed that the oxygen dissolved in the plasma behaves as though it were in combination with the haemoglobin. The error thus introduced is negligible.

[22] Krogh and Lindhard: On the average composition of the alveolar air and its variations during the respiratory cycle. Jour. Physiol., 1914, xlvii, 6, 431–445.

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elimination of carbon dioxide and absorption of oxygen during the time necessary to make a complete expiration. The Haldane-Priestly alveolar samples do not, therefore, represent the true mean alveolar air tension. As near as can be judged from the curves given by Krogh and Lindhard for work in the region of 700 cc. oxygen consumption, the correction would be of the order of −1.0 mm. We have thought it best to make this arbitrary correction as it agrees with the results of some investigations that we have at present under way, though not yet ready for publication.

VII. Respiratory quotient. From the respiration experiments preceding the circulation experiments proper it is evident that the respiratory quotient increases from an average of about 0.8 at rest to about 0.9 at work requiring 900 cc. of oxygen consumption. This increase in the respiratory quotient may have an important metabolic significance or it may be due to a readjustment of the total amount of carbon dioxide stored in the body tissues. We will not now enter into a discussion of this point, as our data are not sufficient to determine such a fundamental process. For this paper it is of importance merely as providing a convenient method of calculating the carbon dioxide elimination per minute under various amounts of oxygen consumption. This is done by multiplying the oxygen consumption by the respiratory quotient.

VIII. The venous carbon dioxide tension. Christiansen, Douglas and Haldene[23] have recently determined the absorption curve of carbon dioxide for human blood and the variations produced on the carbon dioxide carrying capacity by the percentage saturation of the haemoglobin. In Figure 3 of their paper are given the curves for the absorption of carbon dioxide by the blood of J. S. H. in the presence of air (haemoglobin saturated) and in the presence of hydrogen (haemoglobin desaturated). They assume that the line *AB* represents the absorption of carbon dioxide by the blood of J. S. H. within the body at rest on the ground that "if the blood were completely reduced and

[23] Christiansen, Douglas and Haldane: The absorption and dissociation of carbon dioxide by human blood. Jour. Physiol., 1914, xlviii, 4, 244–271.

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a corresponding formation of carbon dioxide occurred, the rise of carbon dioxide would only amount to 22 mm." We reproduce their figure in our Figure V with the addition of lines interpolated to indicate the probable effect of the haemoglobin at 50, 55, 60, 65, 70, and 94 per cent saturation. The formation of the line $A'B'$ will be described presently.

The venous carbon dioxide tension, with allowance for the effect of the percentage saturation of the haemoglobin, can be determined in the following way. For an oxygen consumption of 500 cc. the alveolar carbon dioxide tension is 39.35 mm. (Curve VI, Fig. IV) at which tension the blood contains 51.4 volumes of carbon dioxide (Fig. V, haemoglobin 94 per cent sat.). The respiratory quotient is 0.845 (Curve VII, Fig. IV), therefore the carbon dioxide elimination per minute is $500 \times 0.845 = 423$ cc. per minute. The blood flow per minute is 6.26 L. (Curve I, Fig. IV), therefore each litre of blood carries to the lungs for elimination $\frac{423}{6.26} = 67.6$ cc. of carbon dioxide. In consequence each litre of the venous blood must have contained $51.4 + 6.76 = 58.16$ volumes. As the haemoglobin is 58.7 per cent saturated (Curve V, Fig. IV), the tension at which the carbon dioxide must be for the blood to contain 58.16 volumes is found from Figure V to be 50.1 mm.

In Figure V the place at which the line representing the volume of carbon dioxide absorbed by the blood crosses the line representing the percentage saturation of the haemoglobin has been marked with a large black dot. The points representing the various amounts of oxygen consumption have been connected by the line $A'B'$. The line $A'B'$ therefore represents the absorption of carbon dioxide within the body of W. M. B. under a progressively increasing oxygen consumption per minute; it starts at point A' instead of point A, as the alveolar carbon dioxide tension of W. M. B. is 38 mm. instead of 40 mm. as is the case for Dr. Haldane. The first part of the line $A'B'$ is nearly parallel to the line AB; it then quickly curves to the right and crosses the line AB. Christiansen, Douglas, and Haldane predict that under conditions of work there will be a displace-

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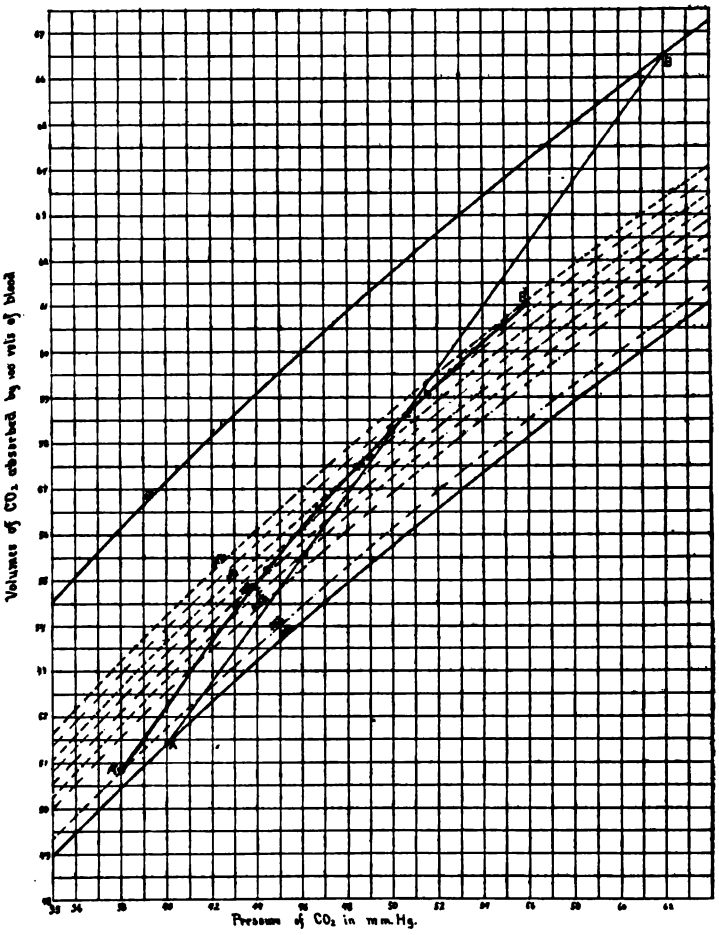


Fig. V. Enlarged section of Figure 3 from paper of Christiensen, Douglas, and Haldane, Jour. Physiol., 1914, xlviii, 4, p. 259. "The upper solid line represents absorption of CO_2 by blood of J. S. H. in presence of hydrogen (Hgb. desaturated). Lower solid line represents absorption of CO_2 by blood of J. S. H. in presence of air (Hgb. saturated). The line *AB* represents the absorption of CO_2 by the blood of J. S. H. within the body." The intermediate dotted lines are interpolated by us to represent the partial degrees of saturation of the haemoglobin with O_2. The line *A'B'* represents the absorption of CO_2 by the blood of W. M. B., as determined from our experiments. Ordinate represents volumes of CO_2 reduced to 760 mm. and 0° dry, absorbed by 100 volumes of blood at 37°. Abscissa represents the pressure of CO_2 in mm. of Hg.

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ment of the line to the right by saying that "during any exertion the difference between arterial and venous carbon dioxide pressure appears to increase very greatly."

The curve representing the venous carbon dioxide tension, however, can only be considered as approximately correct as the absorption curves for carbon dioxide have never been determined for the blood of W. M. B. The error is probably not great and would be of the same order throughout the series.

IX. Hydrogen ion concentration of the arterial blood. In 1905 Haldane and Priestly[24] showed that under normal conditions the breathing is so regulated as to maintain in each individual a practically constant partial pressure of carbon dioxide in the alveolar air. So delicate is the regulation that, other things being equal, a rise of 1 mm. of carbon dioxide pressure increases the breathing by about 80 per cent. Boycott and Haldane[25] in 1908 showed that the action on the respiratory centre of the circulating blood is due to what they called its "total acidity," comprising the free carbon dioxide and some other acid, presumably lactic acid. Hasselbalch[26] has recently shown that these combined acids affect the centre according to the concentration of the hydrogen ions which they produce in the arterial blood.

Campbell, Douglas and Hobson[27] have recently reviewed the earlier experiments of Haldane and Priestly and of Campbell, Douglas, Haldane and Hobson[28] and have carried out further and more exact experiments. They find that "the rise of alveo-

[24] Haldane and Priestly: The regulation of the lung ventilation. Jour. Physiol., 1905, xxxii, 225–266.

[25] Boycott and Haldane: The effects of low atmospheric pressures on respiration. Jour. Physiol., 1908, xxxvii, nos. 5 and 6, 355–377.

[26] Hasselbalch: Neutralitatsregulation und Reizbarkeit des Atemzentrums in ihren Wirkungen auf die Kohlensaurespannung des Blutes. Biochem. Ztschft. 1912, xlvi, 404–439.

[27] Campbell, Douglas, and Hobson: The sensitiveness of the respiratory centre to carbonic acid, and the dead space during hyperpnoea. Jour. Physiol. 1914, xlviii, 4, 303–316.

[28] Campbell, Douglas, Haldane, and Hobson: The response of the respiratory centre to carbonic acid, oxygen, and hydrogen ion concentration. Jour. Physiol., 1913, xlvi, 301–318.

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lar carbon dioxide pressure, (other acids remaining constant), required to give a rise of 10 litres in the alveolar ventilation was 2.5 mm. and in the total ventilation 2.0 mm.; that is, a rise in the carbon dioxide percentage in the dry alveolar air of 0.35 per cent and 0.28 per cent. Judging from Hasselbalch and Lundsgaard's results these figures would imply a rise in the C_H in the arterial blood of about 0.0163×10^{-7} and 0.013×10^{-7} respectively."

These figures obtained in the experiments of Campbell, Douglas, and Hobson are in close agreement with those obtained in earlier experiments with Haldane. The subject (W. M. B.) of the circulation experiments here reported was one of the subjects from which this average figure was obtained by Campbell, Douglas, Haldane, and Hobson. Therefore the sensitiveness of the respiratory centre of W. M. B. is known to agree closely with the figures given above.

Such being the case we can construct Curve IX of Figure IV to represent the hydrogen ion concentration of the arterial blood by dropping a perpendicular line from point *A* on Curve III, which represents an increase of 10 litres in the total ventilation, and running Curve IX through point *B* which is 2.0 mm. above the resting alveolar carbon dioxide tension. The difference between Curves VI and IX is a measure in terms of carbon dioxide, of the amount of lactic acid in the blood under various degrees of work. It is to be noted that severe work, such as could be maintained only for a brief period, was not performed. Work causing an oxygen consumption of 900 cc. was, however, the maximum that could be maintained with reasonable comfort for one and one-half hours; at the end the subject was glad to be through the experiment but was in no way exhausted or "all in."

X. Venous oxygen tension. The tension at which the oxygen will be present for a given percentage saturation, will depend on the total acidity of the blood. The total acidity of the venous blood can be estimated by taking the carbon dioxide equivalent of the lactic acid, found by subtracting points on Curve VI from corresponding points on Curve IX, and adding

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it to corresponding points on Curve VIII. Knowing the total acidity the oxygen tension of the venous blood for any degree of saturation can be read directly from the dissociation curve for oxygen given in Figure VI. Thus for 500 cc. oxygen consumption the difference between Curves VI and IX, representing the lactic acid, is 0.35 mm. The corresponding point on Curve VIII, for the venous carbon dioxide tension, is 50.1 mm. and the total acidity is, therefore, 50.1 + 0.35 = 50.45 mm. The percentage saturation of the haemoglobin for 500 cc. oxygen consumption is 58.7 per cent (Curve V, Fig. IV) and the oxygen tension corresponding to 50.45 mm. of carbon dioxide at this percentage saturation is 32.2 mm.

No direct determinations of the oxygen dissociation curves of Boothby's blood have been made except at 40 mm. carbon dioxide pressure. At this carbon dioxide pressure it is known, however, to agree quite closely with that of Barcroft's blood.[29] Barcroft[30] gives the dissociation curves for his own blood at 20, 40, and 90 mm. carbon dioxide pressure. In Figure VI we have reproduced his figure, enlarged the scale somewhat, and interpolated dotted lines to approximately indicate the intermediate carbon dioxide tensions. The interpolation, though not absolutely correct, is sufficiently accurate for the comparative purposes of this paper.

The heavy line AB in Figure VI represents the curve of oxyhaemoglobin in the blood of W. M. B. within the body and is constructed by connecting the points made by the crossing of the lines representing the percentage saturation with those representing the total acidity of the venous blood in terms of carbon dioxide. It shows that the influence of the total acidity of the blood within the body is of considerable moment and of a distinctly larger order than is thought probable by Christiansen, Douglas, and Haldane. The discrepancy is accounted for by the fact that their calculation, (shown in their Fig. 4), is merely based on one point which was determined at rest, while ours is

[29] Barcroft: The respiratory function of the blood. 1914, University Press, Cambridge, England, p. 219.

[30] Barcroft: loc. cit., p. 65, fig. 34.

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founded on data obtained not only at rest but also with progressively increasing amounts of work. It is possible that even a greater change in the dissociation curve of oxyhaemoglobin would be produced under conditions of prolonged oxygen want such as exists at high altitudes.

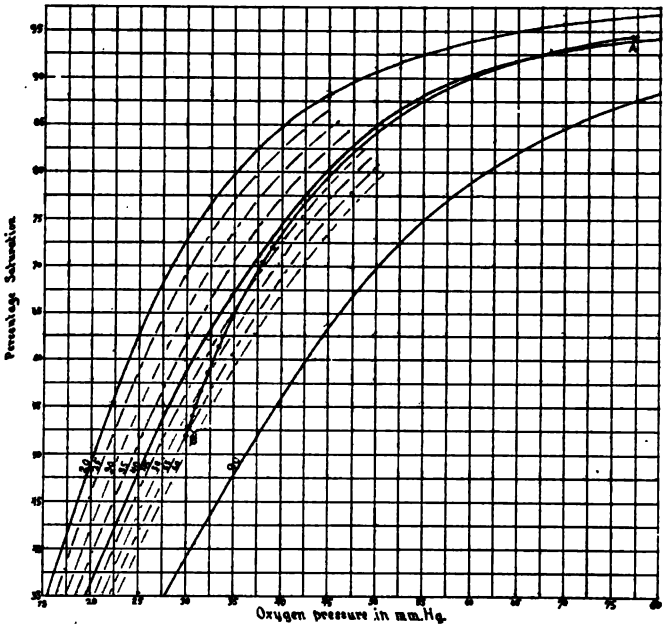


Fig. VI. Enlarged section of Figure 34, p. 65 in Barcroft's book on The Respiratory Function of the Blood. The heavy lines represent the dissociation curve of Barcroft's blood at 20, 40, and 90 mm. CO_2. The dotted lines are interpolated by us to represent the probable effect of the intermediate CO_2 tensions. The line *AB* represents the dissociation curve of the haemoglobin of the blood of W. M. B. within the body. Ordinate represents percentage saturation. Abcissa represents oxygen pressure.

XI. Oxygen pulse. Henderson and Prince[81] have recently introduced the very useful term "oxygen pulse" to represent the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart. Curve XI in Figure IV repre-

[81] Henderson and Prince: The oxygen pulse and the systolic discharge. Amer. Jour. Physiol., 1914, xxxv, 106–115.

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sents the oxygen pulse in our series of experiments and is obtained by dividing the oxygen consumption per minute by the pulse rate.

From the oxygen pulse Henderson and Prince attempt to estimate the probable systolic discharge by computing on the basis "that the heart under normal conditions obeys the principle of 'superimposability of the volume curve.'" On this assumption values are obtained which are quite at variance with ours. The curves for "superimposability" were obtained on dogs and cats with the thorax open and with the animals at rest. To us it does not seem permissible to extrapolate these curves to conditions of severe work and then make use of them for calculating the systolic output of the heart of normal man.

Although Henderson's[32] calculated results for the systolic output are so divergent from ours, as well as from those of Nicolai and Zuntz,[33] yet the experimental data[34] itself appears consistent with our findings, if it is realized that the increase in the heart rate was due in his experiments to other causes than that of increased work with the development of the need of a greater gaseous carrying capacity of the blood per minute.

DISCUSSION OF RESULTS

The experimental evidence here offered shows that the circulation rate increases progressively with the oxygen consumption per minute in a manner corresponding to the increase in the total ventilation.

Respiration consists of various factors the chief of which are: (1) the ventilation of the lungs; (2) the passage of gases through the lung epithelium to and from the blood; (3) the transportation of gases to and from the tissues; (4) cell respiration.

[32] Henderson: The law of the systolic discharge. Internat'l Physiolog. Congress, Vienna, 1910.

[33] Nicolai and Zuntz: Fullung und Entleerung des Herzens bei Ruhe und Arbeit. Berl. klin. Wchusch, 1914 (Nov. 18).

[34] Henderson: The volume curve of the ventricles of the mammalian heart and the significance of this curve in respect to the mechanics of the heart beat and filling of the ventricles. Amer. Jour. Physiol., 1906, xvi, 325–366.

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The respiratory function of the lungs and of the blood is absolutely identical in a true physiological and biological sense. On account of the physical and mechanical problems involved there must, however, be two distinct carrying devices provided for getting the gases from the air to and from the cells.

It is difficult to believe that one part of this respiratory mechanism—the ventilation of the lungs—is carefully and delicately regulated to the needs of the body without the other mechanism—the circulation of the blood—being likewise as carefully and delicately regulated.

It is further highly probable that the same factor which regulates and controls respiration governs both these mechanical processes—the ventilation of the lungs and the circulation of the blood—and not merely one of them as heretofore believed.

On these grounds we feel justified in assuming that the same regulatory factor—the hydrogen ion concentration of the arterial blood—controls with equal delicacy the ventilation of the lungs and the rapidity of the circulation rate.

This conception is strengthened by the evidence given in a previous article[35] in which we showed that after forced breathing —which produces a reduction in the arterial carbon dioxide tension with a parallel decrease in the hydrogen ion concentration—the circulation rate is slowed.

If we assume that the same regulatory factor governs both processes, it is only necessary to show a correspondence between the two processes to estimate the delicacy and sensitiveness of what may be looked upon as a "centre" governing the circulation rate. From what has been said above, it is possible that the "circulatory centre" is really only a part of the respiratory centre. Also that the centre hithertofore considered as the respiratory centre is best considered as a ventilation centre. The respiratory centre can then be thought of as a large centre composed of subordinate centres of which we consider here only the sub-centres controlling pulmonary ventilation and circulation rate.

[35] Boothby: Absence of apnoea after forced breathing. Jour. Physiol., 1912, xlv, 5, 328–337.

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In addition to the chemical regulation of the circulation it is evident that it can be influenced by factors of nervous or psychic origin in the same manner as in the pulmonary ventilation. Our experiments indicate that such influences, however, are only temporary and designed to meet sudden emergencies which require immediately in the muscles a greatly increased oxygen supply before a sufficient time could elapse for the chemical stimulus to be produced and to take effect.

SUMMARY

I. The experimental details as used by us are given for determining the blood flow according to the method of Krogh and Lindhard.

II. A series of sixty-one determinations of the blood flow in one subject at rest and at various degrees of work are reported.

III. By these experiments it is shown that the circulation rate increases proportionately with the oxygen consumption in a manner corresponding to the increase in the total ventilation.

IV. It is suggested that the main controlling factor in the regulation of the circulation rate is the hydrogen ion concentration of the arterial blood and that this regulation is one of great delicacy.

V. By comparing the increase in the circulation rate with the increase in the total ventilation we are able to estimate that on the subject studied an increase in the blood flow of 3.3 litres per minute, which is a doubling of the circulation rate, is caused by a rise in the total acidity of the blood corresponding to 2.0 mm. of carbon dioxide. As pointed out by Campbell, Douglas, and Hobson, it is possible to deduce from the results of Hasselbalch and Lundsgaard that this figure would correspond to a rise in the hydrogen ion concentration of the arterial blood of about 0.013×10^{-7}.

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TABLE II

Circulation experiments—subject lying at rest

EXPERIMENT NO.	DATE	ELEMENTS OF EXPERIMENT																BLOOD FLOW DATA				VALUES REDUCED TO NORMAL EXCHANGE			
								Spirometer experiment					Sample I			Sample II									
		1 Bar. (corr.)	2 Temp. spir.	3 Length introd. period	4 Length experiment period	5 Initial volume	6 Final volume	7 CO_2 per min.	8 O_2 per min.	9 R. Q. $\frac{7}{8}$	10 Ventilation per min. at 37°	11 Pulse	12 O_2	13 N_2O	14 N_2	15 O_2	16 N_2O	17 N_2	18 O_2 absorbed per min.	19 Blood flow per min.	20 Pulse	21 Vol. per beat $\frac{18}{19}$	22 Blood flow per min.	23 O_2 per litre blood $\frac{8}{22}$	24 Vol. per pulse beat $\frac{21}{11}$
				secs.	*secs.*	*litres*	*litres*	*cc.*	*cc.*		*litres*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*litres*		*cc.*	*litres*	*cc.*	*cc.*
1	May 6	750.3	20.8°	10.1	20.2	2.38	2.28	150	183	0.82	5.78	57	14.13	13.42	67.79	11.66	11.47	70.70	175	3.30	52.2	63	3.45	53	61
2	May 6	750.3	20.8°	12.0	23.7	2.01	1.90	150	183	0.82	5.78	57	13.63	12.00	69.25	10.50	9.92	73.40	168	2.90	51.4	56	3.16	58	55
3	May 7	756.5	21.8°	11.2	21.0	2.31	2.20	136	159	0.86	5.24	56	14.29	12.86	68.10	11.97	10.45	71.62	171	3.90	52.6	74	3.63	44	65
4	May 7	756.5	21.8°	11.5	23.8	2.50	2.37	136	159	0.86	5.24	56	14.01	13.42	67.37	11.51	11.37	71.09	175	3.19	51.3	62	2.90	55	52
5	May 9	752.9	21.8°	9.7	19.8	2.10	2.00	161	189	0.85	6.03	61	14.56	12.41	68.36	11.77	10.32	71.63	190	3.43	57.4	60	3.41	55	56
6	May 9	752.9	21.8°	9.4	19.7	1.89	1.80	161	189	0.85	6.03	61	14.35	12.33	68.65	11.52	10.06	72.11	174	3.36	56.5	59	3.65	52	60
7	May 9	752.5	21.8°	10.4	19.6	2.27	2.17	140	171	0.82	5.01	58	14.52	13.05	68.06	12.14	10.81	71.09	181	3.75	56.0	67	3.54	48	61
8	May 9	752.5	21.8°	12.7	18.3	1.97	1.88	140	171	0.82	5.01	58	14.22	13.16	68.06	11.65	11.00	71.37	179	3.38	55.8	61	3.21	53	55

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TABLE III

Circulation experiments—subject sitting at rest

EXPERIMENT NO.	DATE	ELEMENTS OF EXPERIMENT																	BLOOD FLOW DATA				VALUES REDUCED TO NORMAL EXCHANGE		
								Spirometer experiment					Sample I			Sample II									
		Bar. (corr.)	Temp. spir.	Length introd. period	Length experiment period	Initial volume	Final volume	CO_2 per min.	O_2 per min.	$\frac{R}{I}$ R. Q.	Ventilation per min. at 37°	Pulse	O_2	N_2O	N_2	O_2	N_2O	N_2	O_2 absorbed per min.	Blood flow per min.	Pulse	$\frac{13}{12}$ = 21	Blood flow per min.	O_2 per litre blood $\frac{7}{21}$ = 23	Vol. per pulse beat $\frac{11}{22}$ = 24
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
				secs.	*secs.*	*litres*	*litres*	*cc.*	*cc.*		*litres*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*litres*		*cc.*	*litres*	*cc.*	*cc.*
1	Mar. 25	755.8	18.8°	8.0	24.0	3.32	3.24		(185)				16.27	6.90	72.98	13.83	5.93	74.81	215	3.46			(2.98)	(62)	
2	Mar. 25	755.8	18.8°	7.2	21.0	3.03	2.96		(185)				15.82	9.40	71.03	13.92	8.08	72.81	180	3.59			(3.69)	(50)	
3	Mar. 26	767.6	18.9°	9.8	21.1	3.03	2.95		(185)				15.30	9.10	71.42	13.44	8.00	73.32	176	3.18			(3.34)	(55)	
4	Mar. 30	761.5	19.1°	10.2	20.2	2.81	2.75		(185)				15.96	8.06	72.24	13.81	6.82	73.83	187	3.74			(3.70)	(50)	
5	Mar. 30	761.5	19.1°	9.8	20.6	2.23	2.15		(185)				15.54	8.89	71.46	12.73	7.40	73.99	195	3.38			(3.21)	(58)	
6	Apr. 3	755.7	16.9°	8.0	22.1	2.34	2.21		(185)				15.04	11.11	69.72	11.00	8.85	73.90	271	4.26			(2.91)	(64)	
7	Apr. 3	755.7	16.9°	10.3	17.6	2.73	2.65		(185)				15.15	10.32	70.40	12.51	9.29	72.46	257	3.00			(2.16)	(86)	
8	Apr. 8	757.8	18.1°	7.8	25.0	2.85	2.72		(185)				15.88	9.94	70.53	12.72	7.69	73.85	234	4.88			(3.86)	(48)	
9	Apr. 9	752.7	21.2°	11.2	19.6	3.21	3.15		(185)				14.64	12.72	68.63	12.59	12.08	69.91	198	1.61	56.7	28	(1.50)	(123)	
10	Apr. 10	761.5	19.5°	10.4	13.9	2.80	2.72		(185)				14.61	12.99	68.46	13.33	10.75	70.57	183	5.55	55.0	101	(5.61)	(33)	
11	Apr. 10	761.5	19.5°	9.7	13.3	2.67	2.61		(185)				14.93	11.92	69.10	13.57	10.54	70.70	183	4.15	54.5	76	(4.20)	(44)	
12	Apr. 14	768.2	21.5°	10.2	17.8	2.44	2.35		(185)				14.97	11.17	69.78	12.65	9.20	72.51	209	4.42	55.9	79	(3.91)	(47)	
13	Apr. 14	768.2	21.5°	9.0	18.9	2.32	2.21		(185)				14.66	13.23	68.09	12.08	10.80	71.34	211	4.27	56.1	76	(3.74)	(49)	
14	Apr. 15	766.9	20.5°	10.4	22.4	2.36	2.26		(185)				14.11	13.56	68.07	11.70	11.30	71.23	168	3.32	55.9	59	(3.66)	(51)	
15	Apr. 15	766.9	20.5°	10.0	21.9	2.31	2.19		(185)				14.32	12.82	68.48	11.39	10.48	72.16	203	3.73	59.7	62	(3.40)	(54)	
16	Apr. 15	766.9	20.5°	10.1	20.8	2.35	2.25		(185)				14.15	13.48	67.97	11.19	11.61	71.10	213	3.04	59.3	51	(2.64)	(70)	
17	Apr. 16	751.6	19.6°	9.0	24.1	2.15	2.01		(185)				14.30	13.41	68.01	11.04	10.05	72.72	192	4.40	50.2	88	(4.24)	(44)	
18	Apr. 17	757.8	19.6°	9.7	21.3	2.50	2.36		(185)				14.19	14.42	67.05	11.38	11.48	71.05	221	4.67	58.7	80	(3.91)	(47)	
19	Apr. 17	757.8	19.6°	9.9	22.6	2.51	2.37		(185)				14.38	14.15	67.25	11.56	11.40	71.09	210	4.23	57.1	74	(3.73)	(50)	
20	Apr. 22	759.1	22.0°	10.2	23.2	2.58	2.47	149	189	0.79	6.75	59	14.50	13.99	67.53	12.33	11.42	70.66	162	3.80	54.8	69	4.43	43	75
21	Apr. 22	759.1	22.0°	10.5	25.0	2.51	2.38	149	189	0.79	6.75	59	14.29	15.69	66.18	11.92	12.86	69.66	162	3.49	54.0	65	4.07	46	69
22	Apr. 23	765.8	21.7°	10.0	24.2	2.52	2.41	136	176	0.77	6.30	58	14.54	11.98	69.01	12.14	9.80	72.20	167	3.55	52.2	68	3.74	47	64
23	Apr. 23	765.8	21.7°	11.4	25.1	2.53	2.42	136	176	0.77	6.30	58	14.27	13.85	67.66	12.11	11.56	70.89	148	3.16	48.5	65	3.76	47	65
24	Apr. 23	765.8	21.7°	10.4	24.6	2.59	2.49	136	176	0.77	6.30	58	14.45	13.54	67.94	12.23	11.61	70.79	154	2.83	50.5	56	3.23	55	56
25	Apr. 24	767.4	20.5°	9.5	25.5	2.66	2.54	148	188	0.79	6.96	58	14.58	13.23	68.09	12.26	11.07	71.21	165	3.27	53.8	61	3.73	50	64
26	Apr. 24	767.4	20.5°	10.3	21.1	2.50	2.40	148	188	0.79	6.96	58	14.10	13.62	67.94	11.96	11.75	70.83	169	3.13	53.3	59	3.48	54	60

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TABLE IV

Circulation experiments—subject working

EXPERIMENT NO.	DATE	ELEMENTS OF EXPERIMENT																BLOOD FLOW DATA				VALUES REDUCED TO NORMAL EXCHANGE			
								Spirometer experiment					Sample I			Sample II									
		Bar. (corr.)	Temp. spir.	Length introd. period	Length experiment period	Initial volume	Final volume	CO_2 per min.	O_2 per min.	R. Q.	Ventilation per min. at 37°	Pulse	O_2	N_2O	N_2	O_2	N_2O	N_2	O_2 absorbed per min.	Blood flow per min.	Pulse	Vol. per beat	Blood flow	O_2 per litre blood	Vol. per pulse beat
		1	2	3	4	5	6	7	8	$\frac{7}{8}=9$	10	11	12	13	14	15	16	17	18	19	20	$\frac{19}{20}=21$	22	$\frac{18}{19}=23$	$\frac{19}{11}=24$
				secs.	*secs.*	*litres*	*litres*	*cc.*	*cc.*		*litres*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*litres*		*cc.*	*litres*	*cc.*	*cc.*
1	June 4	758.6	21.0°	10.1	20.8	2.50	2.35	225	284	0.79	9.24	65	22.62	13.67	59.76	18.86	11.57	63.50	319	3.81	57.6	66	3.39	84	52
2	June 4	758.6	21.0°	9.9	17.8	2.30	2.18	225	284	0.79	9.24	65	19.59	13.88	62.63	16.07	11.95	66.02	306	3.65	58.1	63	3.39	84	52
3	June 5	758.6	20.0°	9.1	24.2	2.69	2.50	261	313	0.83	10.0	81	16.25	16.51	62.88	13.54	13.36	67.78	279	4.38	63.7	69	4.91	64	61
4	June 5	758.6	20.0°	10 3	24.3	2.62	2.46	261	313	0.83	10.0	81	17.07	14.31	64.31	13.49	11.79	68.52	259	3.85	62.7	61	4.65	67	57
5	June 10	769.3	20.5°	10.6	25.1	2.77	2.58	270	322	0.84	10.0	72	17.78	13.80	63.83	13.54	11.29	68.49	314	4.14	59?	70?	4.25	76	59
6	June 10	769.3	20.5°	10.2	23.3	2.49	2.32	270	322	0.84	10.0	72	17.53	14.38	63.57	13.52	11.75	68.21	290	4.01	54?	74?	4.45	72	62
7	June 2	762.1	21.5°	10 7	18.0	2.49	2.35	277	328	0 84	10.1	86	13.73	15.11	66.35	11.16	12.34	70.33	240	4.96	65.1	76	6.78	48	79
8	June 2	762.1	21.5°	9.0	20.5	2.72	2.57	277	328	0.84	10.1	86	13.97	15.57	66.10	11.14	12.71	70.03	248	4.75	68.7	69	6.28	52	73
9	June 2	761.9	23.0°	12.2	19.3	2.55	2.42	269	329	0.82	9.72	78	13.16	14.63	67.30	10.60	12.36	70.83	221	4.02	59.3	68	5.98	55	77
10	June 2	761.9	23.0°	10.4	20.9	2.50	2.36	269	329	0.82	9.72	78	13.87	14.84	66.88	10.55	12.29	70.93	252	4.05	60.0	67	5.29	62	68
11	May 26	761.2	24.0°	9.5	15.6	2.61	2.48	261	331	0.79	9.37	73	14.21	14.26	67.37	11.35	11.80	70.85	307	5.51	59.6	92	5.94	56	81
12	May 29	772.8	21.0°	9.6	24.8	2.61	2.43	258	333	0.78	10.2	68	13.97	14.60	67.22	9.98	11.59	73.34	272	4.38	57.3	76	5.36	62	79
13	June 22	760.5	21.5°	12.3	16.2	2.66	2.50	372	443	0.84	12.2	86	17.35	13.26	64.43	13.32	11.37	68.45	430	4.89	74.0	66	5.04	88	59
14	June 22	760.5	21.5°	9.3	16.1	2.42	2.27	372	443	0.84	12.2	86	18.37	13.08	63.97	14.01	10.92	68.20	426	5.06	75.0	67	5.26	84	61
15	June 11	755.7	23.0°	9.7	18.2	2.61	2.43	388	452	0.86	13.3	87	17.15	13.22	64.64	12.63	10.92	69.38	414	5.14	70.6	73	5.61	81	64
16	June 15	758.1	20.7°	7.0	12.4	2.55	2.40	476	554	0.86	15.5	97	17.77	12.93	64.62	13.43	10.83	68.79	572	6.76	75.0	91	6.58	84	68
17	June 15	758.1	20.7°	8.1	13.5	2.41	2.24	476	554	0.86	15 5	97	19.00	14.29	62.06	14.39	12.01	66.71	544	6.08	79.7	76	6.19	90	64
18	June 18	764.2	22.0°	9.2	11.4	2.39	2.24	494	563	0.88	15.4	94	16.92	12.44	65.21	12.67	10.50	69.48	575	6.76	80.8	84	6.62	85	70
19	June 18	764.2	22.0°	8.7	14.4	2.47	2.29	494	563	0.88	15.4	94	18.20	14.09	62.57	13.64	11.65	67.52	518	6.24	77.4	81	6.78	83	72
20	June 16	754.7	20.5°	8.7	13.2	2.32	2.15	542	608	0.89	16.8	91	17.23	14.06	63.53	12 83	11.45	68.45	507	6.80	78.3	87	8.16	75	90
21	June 16	754.7	20.5°	9.4	12.9	2.72	2.56	542	608	0.89	16.8	91	17.75	13.43	63.55	13.75	11.50	67.60	547	6.31	79.3	80	7.01	87	77
22	June 12	755.1	25.0°	6.7	8.7	2.51	2.34	821	909	0.90	24.5	140	17.83	12.31	64.51	13.08	10.30	69.27	860	9.69			10.24	89	73
23	June 25	755.6	27.8°	6.8	7.4	2.56	2.40	842	912	0.92	24.2	130	17.27	14.04	63.24	13.14	12.07	67.39	893	9.94	133	75	10.15	90	78
24	June 25	755.6	27.8°	5.7	7.9	2.40	2.28	842	912	0.92	24.2	130	17.22	14.22	63.26	12.54	13.36	66.71	838	4.62	127	36	5.03	181	39*
25	June 23	760.6	23.2°	6.1	8.1	2.43	2.29	831	914	0.91	23.1	129	18.00	13.48	63.29	13.55	11.78	67.16	845	8.00	126	64	8.65	106	67
26	June 24	758.9	25.0°	8.8	8.2	2.80	2.66	863	914	0.94	24.4	133	17.27	12.91	64.38	13.15	11.67	67.77	868	7.10	124	57	7.48	122	58
27	June 24	758.9	25.0°	6.5	7.2	2.69	2.54	863	914	0.94	24.4	133	17.49	12.81	64.10	13.76	11.19	67.78	893	9.79	131	75	10.02	91	75

* Exp. 24 omitted in the averages.

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A STUDY OF THE LATE EFFECT OF DIVISION OF THE PULMONARY BRANCHES OF THE VAGUS NERVE ON THE GASEOUS METABOLISM, GAS EXCHANGE, AND RESPIRATORY MECHANISM IN DOGS

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The study of the function of the vagi in the lungs is complicated by their extensive distribution to other organs. To simplify, therefore, the interpretation of the data obtained, we have divided only the pulmonary branches of both vagi. These are the branches given off from the vagi between the recurrent laryngeal and the point where each vagus divides into its two primary gastro-intestinal branches. It is possible, however, that a few fibres of the upper branches have a cardiac instead of a pulmonary destination. Otherwise the distribution and functions of the vagi are left unimpaired.

The interpretation of the results obtained immediately after section of a nerve is always questionable, as the effect produced may be due to a cessation of its influence, or to overactivity from irritation of its divided ends, or indirectly to the general and local effect of the necessary operative procedure.

These difficulties have been avoided by dividing the pulmonary branches with the precautions requisite for intrathoracic surgery and allowing the animals to completely recover. The respiratory studies were made three to four months after the division of the vagi.

For convenience we shall refer throughout this paper to the

[1] Dr. Shamoff was recalled to Russia for military service before this article was completed.

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animals thus prepared as vagotomized dogs, but we wish to emphasize the fact that only the pulmonary, and possibly a few cardiac, branches were divided and that the other branches of the vagi were left intact and their functions therefore were presumably unimpaired. In consequence our data cannot be compared with the results obtained immediately after section of the vagi in the neck.

EFFECT ON HEALTH

Out of the series of eleven dogs operated on we had four permanent recoveries. The cause of death in the unsuccessful experiments was usually a diffuse bronchial pneumonia; less frequently a supperative pleurisy. The pulmonic infection was, however, more frequent after operation on the vagus nerve than after other intrathoracic operations, a series of which were being carried on at the same time. The post-operative recovery was in every way similar to that of animals which had undergone other intrathoracic operations and none of the deaths could be traced to the division of the vagi.

The four animals that recovered quickly became strong and active. Their diet was liberal and consisted of cooked lean meat and bread, and for exercise they were allowed the freedom of an outdoor pen. Our animals were the only dogs living permanently in the animal house during the spring and summer out of a shifting population of about twenty dogs. The vagotomized dogs became strong, active and playful, but they showed no tendency to gain in weight, remaining, in fact, abnormally thin. Furthermore, they were all quite severely affected with the mange. We do not know whether this lean and mangy condition was in any way dependent on an obscure derangement of the metabolic processes caused by the interference with the vagus supply of the lungs.

METABOLISM

The basal respiratory metabolism was determined by means of a Benedict respiration apparatus and all the experimental details were carried out in the manner and with all the precautions

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recommended by Benedict.[2] During the experimental investigation all the animals were living under identical conditions. Before experimentation food was withheld for at least fourteen hours. To record any movements made by the animal during an experiment a pneumograph was passed across the hind quarters. In the last column of Table III we have paraphrased this record.

In order to obtain a graphic curve of the respirations, use was made of an air tight mask over the dog's head, instead of using the calorimeter box described by Benedict. As the application of such a mask must be absolutely air tight, we give in detail the technic finally adopted by us. A Tissot mask[3] fitting snugly over the dog's snout was held in place by straps passing behind the ears. Very thin rubber tissue was placed over the eyes, and about a quarter inch layer of vaseline was spread over the mask, head and neck of the dog. Over this was pulled a long bag made of rubber dental dam which reached to the base of the neck. The whole was firmly bound down by the even application of bandages. The vaseline, pressed down into the hair, made an air tight connection capable of withstanding an air pressure of fifteen centimeters of water.

During the first application of the mask, the dogs were somewhat frightened. Therefore, on the first day we merely connected them to the respiration apparatus without carrying through an actual experiment. After becoming accustomed to the mask and apparatus, it was surprising how quietly the dogs would lie on the padded table for hours at a time without restraint.

To check the results obtained from the Benedict apparatus we determined the respiratory exchange on two of the vagus dogs by the method of collecting, measuring, and analyzing the expired air. The accuracy of the method is essentially a question of the efficiency of the respiratory valves. For this reason,

[2] Benedict: Ein Universalrespirationsapparat. Deutsch. Arch. f. Klin. Med. 1912, cvii, 155–200.

[3] Tissot: Nouvelle method de mesure et d'inscription du debit et des mouvements respiratoires de l'homme et des animaux. Journ. d. Phys. et Path. Gen., 1904, vi, 688–700.

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in addition to the Tissot valves on the mask, we also placed on the inspiratory side a large especially constructed valve, similar to the Douglas valve made by Siebe, Gorman & Co. The technic was essentially that described by Douglas[4] for the determination of the total respiratory exchange in man, excepting that a 30 L. calibrated spirometer was used instead of a bag and meter.

It will be noted that the spirometer experiments show a slightly lower oxygen consumption and carbon dioxide elimination as well as a slightly lower respiratory quotient than do the experiments with the Benedict apparatus. As the experimental pe-

TABLE I

Summary of respiratory exchange experiments

SUBJECT	CARBON DIOXIDE		OXYGEN		RESP. QUO.
	Per min.	Per kilo per min.	Per min.	Per kilo per min.	
	cc.	*cc.*	*cc.*	*cc.*	
Normal Dog					
No. 1.....	33	5.9	44	7.8	0.75
Vagus Dogs					
No. 18.....	93	7.0	123	9.3	0.75
No. 15.....	89	6.9	119	9.2	0.75
No. 23.....	99	6.0	134	8.1	0.74
No. 11.....	94	6.6	114	8.0	0.82

riod extended over four and sometimes five hours, during which time the dog was lying at rest without food or drink, and as the spirometer experiments were the last performed, it is probable that the metabolism was slightly lower than it had been earlier in the day.

In Table III, at the end of the paper, are given the essential data and the calculated results of our experiments on the metabolism of the vagotomized dogs, together with the results obtained on a perfectly normal animal, living under the same conditions, and with the experiments conducted in an identical manner. The data is summarized in Table I in which are given the aver-

[4] Douglas: A method for determining the total respiratory exchange in man. Jour. Physiol., 1911, xlii, Proc. Physiol. Soc., Mar. 18.

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ages of the experiments for each dog. Figure I shows a part of a typical curve obtained in a metabolism experiment on one of the vagotomized dogs (dog No. 15).

From these experiments it is evident that the gaseous metabolism is in no demonstrable way affected by the division of the pulmonary branches of the vagi.

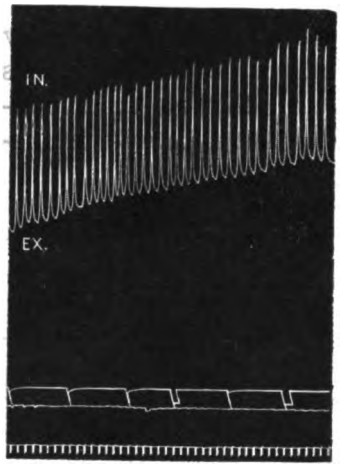


Fig. I.



Fig. II.

Fig. I. Subject, Dog No. 15. Weight, 12.9 kg. No food for fourteen hours. Section of a curve obtained in one of the metabolism experiments on above dog. Upper curve is written by the spirometer on the Benedict respiration apparatus. The second curve is from the work adder, from which the total ventilation is calculated. Third curve is made by a tambour attached to a pneumograph, passing over the dog's hind quarters. This curve shows whether or not the animal was quiet during the experiment. Lower curve is the time in 5 seconds.

Fig. II. Subject, Dog. No. 11. Five days after division of the pulmonary branches of the right vagus (the left had been divided about two weeks previously). Tracing written by a tambour around the chest and is not quantitative. Dog panting. Time in seconds.

RESPIRATORY RHYTHM

Lewandowsky[5] and other observers have noted a marked prolongation of the inspiratory phase after vagotomy. To our surprise no trace of this phenomenon occurred in any of our vagotomized animals.

[5] Lewandowsky: Nagels Hdbk. d. Phys., i, 38.

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Dog No. 11 on withdrawal of the intratracheal tube for insufflation anaesthesia, after the second operation, showed on the table a deep Cheyne-Stokes type of respiration with great rhythmical exertion of all the respiratory muscles, and the expiratory phase was much prolonged. At the time we considered the phenomenon as possibly of vagal origin. The animal recovered from the operation rapidly, and five days later we obtained the tracings reproduced in Figures II and III. Figure II is the respiratory curve made by a tambour fastened around the chest.

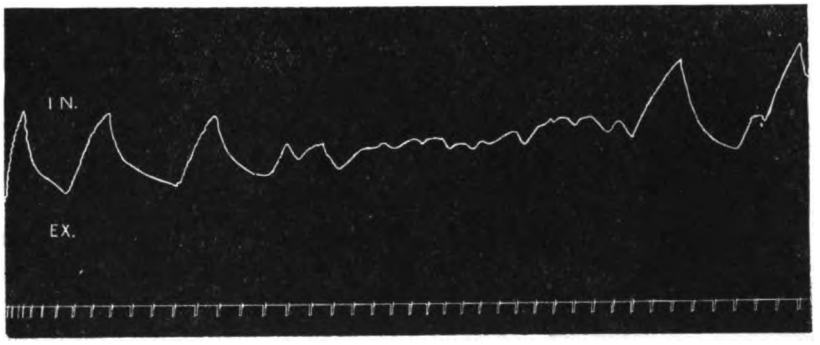


Fig. III. Subject, Dog. No. 11. Curve obtained a few hours after that of Fig. II. Quantitative respiration curve from the Benedict apparatus and shows the irregularity of the respiratory rhythm, resembling the Cheyne-Stokes type. Time in seconds.

Figure III is a spirometer tracing obtained by connecting the animal with the Benedict apparatus, as described above. This breathing shows typical Cheyne-Stokes characters. In every way it resembles the artificial Cheyne-Stokes respiration shown by Haldane and Douglas[6] to be produced in normal persons by breathing through a long tube. It is evident that the changes in the gases of the alveolar air, blood, and ventilation centre, which cause rhythmic breathing, would be produced equally as well by a partial pneumothorax as by breathing through a tube.

In Figure IV is given an example of the respiratory curve obtained from this same animal four months later. Neither in

[6] Haldane and Douglas: The experimental production of Cheyne-Stokes breathing in normal persons. Int. Phys.-Kongr. Wien., 1910, viii.

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this or any of the other tracings is there any evidence of the persistence of the rhythmic Cheyne-Stokes type of respiration. The only possible abnormality is the occasional slight prolongation of the pause at the end of expiration. It is therefore probable that the pneumothorax had by this time entirely disappeared.

Several dogs subsequently operated on showed no signs of this phenomenon, even when branches of both vagi were divided at one stage. Consequently we decided that the division of the vagus was not the cause of the abnormal respiration of dog No. 11. Finally, we had another and even more marked example of this type of respiration as follows: On removal of the intratracheal tube the dog became cyanotic and it was necessary to reintroduce the tube to maintain life. The respiratory exertions of the animal were extreme and it appeared as though the diaphragm and intercostal muscles might not be working synchronously. On opening the abdomen, however, we found that the diaphragm and chest muscles were contracting in perfect unison and to an obviously great extent. As the air-way was free, we were at a loss to explain the phenomenon until it was suggested that a pneumothorax existed; on investigation the lungs were found two-thirds collapsed.

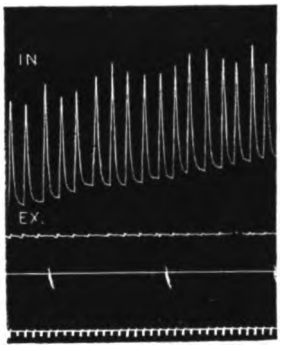


Fig. IV. Subject, Dog No. 11. About four months after obtaining the curves in Figures II and III. It shows possibly a slight and irregular prolongation of the pause at the end of expiration. Time in 5 seconds.

We therefore believe our technic for avoiding pneumothorax in dog No. 11 was faulty and that the Cheyne-Stokes respiration, existing for about a month after the operation, was due to difficult aëration of the lungs from the existence of a partial pneumothorax, and was consequently independent of the vagus operation. The prolongation of the expiratory phase in Figure IV, four months after the operation, is so slight that it cannot be classed as really abnormal.

According to our experiments, the respiratory rhythm is not

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affected by division of the pulmonary branches of the vagi. Our evidence is, therefore, against Meltzer's[7] hypothesis, which is an elaboration of that of Hering and Breuer,[8] that the respiratory rhythm is normally controlled by stimuli created by the alternate expansion and collapse of the lungs and which pass to the respiratory centre over two separate sets of nerves fibres in the vagus trunk. It is to be noted, however, that our experiments are not concerned with the question whether the vagi contain fibres that, on electrical stimulation, will incite or inhibit respiration, but only with the part which they are supposed to play in the normal regulation of respiration.

RESPONSE TO AN INCREASE OF CARBON DIOXIDE AND TO A DECREASE OF OXYGEN IN THE INSPIRED AIR

In the preceding section we have shown that division of the pulmonary branches of the vagi did not demonstrably affect respiration when breathing normal air. To gain still further evidence in regard to the possible functions of the vagi over the respiratory process, we arranged the following experiments.

The soda lime absorber was removed from the Benedict respiration apparatus and a large air container inserted to increase the total volume of air in the apparatus. In consequence, the carbon dioxide in the inspired air gradually increased; the oxygen at the same time decreased somewhat, though not sufficiently to be a material factor in the experiment.

The character of the response to the gradual increase of the carbon dioxide is shown in Figure V, together with the analyses of the inspired air samples obtained at the points indicated.

These experiments confirm the findings of Haldane and Lorrain Smith[9] that the stimulation of the vagus nerve endings by carbonic acid has nothing to do with hyperpnoea.

[7] Meltzer: The self-regulation of respiration. N. Y. Med. Jour., 1890, li, Jan. 18; lii, Nov. 22.

[8] Hering and Breuer: Die Selbsteuerung der Athmung durch den Nervus Vagus. Sitzgsber. d. Wiener Acad., Math.-natur., 1868, lvii, 672; lviii, 909.

[9] Haldane and Lorrain Smith: The physiological effects of air vitiated by respiration. Jour. Path. and Bact., 1892, i, 168–186.

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The effect of oxygen want was tested by introducing air instead of oxygen into the Benedict apparatus to maintain a constant air volume, and at the same time to lower gradually the oxygen percentage (the carbon dioxide being absorbed as usual). Under these conditions the respirations increased quite markedly in rate but only slightly in depth, as shown by Figure VI. A more pronounced effect is shown in Figure VII, where the oxygen was low throughout the experiment. The effect of

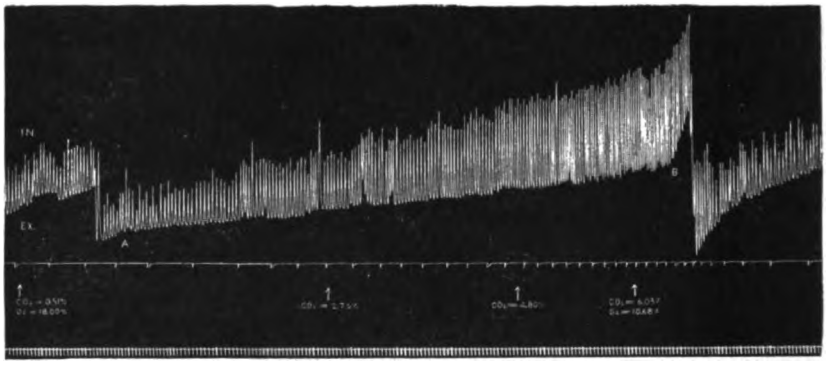


Fig. V. Subject, Dog No. 18. Upper curve is the spirometer tracing showing reaction to the gradual increase of CO_2 in the inspired air. The middle curve is made by the work adder and shows the total ventilation. Lower curve is the time in 5 seconds. Samples of the inspired air were taken at the points indicated. (1) CO_2, 0.51 per cent; O_2, 18.00 per cent; (2) CO_2, 2.73 per cent; (3) CO_2, 4.80 per cent; (4) CO_2, 6.03 per cent; O_2, 10.68 per cent. At point *A* the tap on the apparatus was turned to allow the CO_2 to accumulate; at *B* the tap was turned so that the CO_2 was absorbed. The change in the level of the tracing before *A* is due to adding O_2. The change upwards at *B* is due to the absorption of CO_2; and the change downwards to a rapid addition of O_2. These changes in the level of the curve have no significance as far as the subject is concerned.

oxygen want, as shown in these two figures, is unlike that obtained from the rise in the alveolar carbon dioxide pressure, as shown by Figure V, where the depth of the respiration is more markedly increased than the rate.

The curves here shown of the effect of carbon dioxide increase and oxygen want are in every way similar to those obtained by Haldane and others for the effect of such conditions in man; they are similar to the curves obtained by us on a normal dog.

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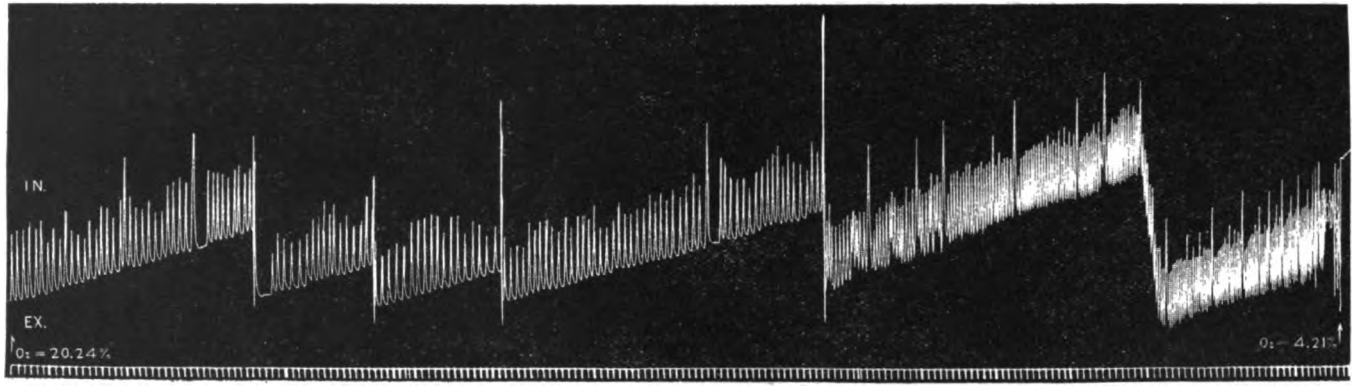


Fig. VI. Subject, Dog No. 18. Shows reaction to a gradual decrease in the O_2 in the inspired air from 20.24 per cent at the beginning to 4.21 per cent at the end. Time in 5 seconds.

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It is possible, therefore, to conclude that the vagus nerve does not contain fibres which transmit impulses arising from variations in the composition of the alveolar air.

INFLUENCE OF THE VAGUS ON THE SECRETORY POWER OF THE LUNGS

Maar[10] has described experiments which, he believed, showed evidence of the influence of the vagus nerve on the gas secretion of the lungs. Krogh[11] later pointed out that the results obtained by Maar could be explained by a vasomotor, instead of a direct secretory influence.

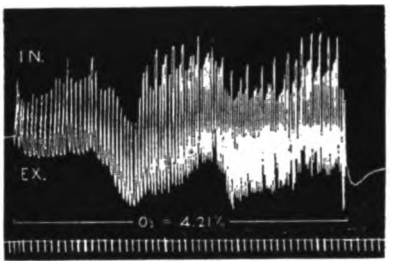


Fig. VII. Subject, Dog No. 18. This curve obtained about twenty minutes after the curve in Fig. VI. It shows the reaction on being suddenly made to breathe air containing only 4.21 per cent of O_2. Time in 5 seconds.

The metabolic experiments already cited indicate that there is no impairment of the gas exchange function of the lungs. However, as Haldane and Douglas[12] only claim a secretory action in a condition of oxygen want, we arranged a few experiments to test the effect on the oxygen intake under conditions of oxygen want.

The Benedict apparatus was arranged with an extra container to enlarge the total volume of air in the apparatus, and air (carbon dioxide free), was used instead of oxygen to maintain a constant volume. In other respects the metabolic experiments were conducted as those described previously. The analyses of the inspired air at the beginning and at the end of the experiments, together with the metabolic data, are given in Table II.

[10] Maar: Exp. Untersuchungen uber den Einfluss des Nervus Vagus und des Nervus Sympathicus auf den Gaswechsel der Lungen. Skand. Arch. f. Physiol., 1902, xiii, 269–336.

[11] Krogh: On the mechanism of the gas exchange in the lungs of the tortoise. Skand. Arch. f. Physiol., 1909, xxiii, 200–216.

[12] Haldane and Douglas: The causes of the absorption of oxygen by the lungs. Jour. Phys., 1912, xliv, 4, 305–354.

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From these experiments it is evident that in spite of the low oxygen tension in the inspired air (about 30 mm.), the animals absorbed as much oxygen per minute as when breathing pure air. In fact, they showed in several instances a distinctly greater intake. This is due to the fact that the very low oxygen tension, necessarily existing in the blood, caused the animals to be somewhat restless. In the region of the belly it was easily seen that the blood was quite cyanotic.

TABLE II

Respiratory exchange under conditions of oxygen want

DATE	VAGUS DOG	WEIGHT DOG	EXPER. NO.	PREVIOUS MEAL		CARBON DIOXIDE		OXYGEN		RESP. QUO.	OXYGEN PER CENT	
				Time	Character	Per min.	Per kilo per min.	Per min.	Per kilo per min.		Start	End
		kilos				*cc.*	*cc.*	*cc.*	*cc.*		*per cent*	*per cent*
Aug. 24	No. 18	13.2	1	8/23; 12 n.	Meat diet largely	100	7.6	120	9.1	0.84	20.24	4.21
Oct. 7	No. 11	14.2	1	10/6; 3.30 p.m.	Meat diet largely	104	7.3	114	8.0	0.91	24.71	11.14
Oct. 7	No. 11		2	3.30 p.m.	Meat diet largely	92	6.5	95	6.7	0.96	16.42	15.18
Oct. 7	No. 11		3	3.30 p.m.	Meat diet largely	116	8.2	112	7.9	1.04	20.03	5.29
Oct. 7	No. 11		4	3.30 p.m.	Meat diet largely	127	8.9	130	9.2	0.98	12.51	6.41
						110	7.7	113	8.0	0.97		

These experiments throw no direct evidence on the question of gas secretion as opposed to the theory of simple diffusion. They do, however, indicate that the process of gas exchange is not interfered with by the cessation of its vagus nerve supply. It is fair to presume that if the lungs were a true secretory organ for oxygen, interference with the nerve supply would result in a greater impairment of the function than if the gas passed through the organ according to the physical laws of diffusion.

Therefore, as the passage of oxygen through the lungs, even at a low tension, is apparently not interfered with by the division of the vagi, the evidence offered is against the assumption of a

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secretory function of the lungs, or at least, it indicates that if such a function does exist the vagi do not govern the secretory action.

SUMMARY

All the branches of both right and left vagi between the recurrent laryngeal and the two primary gastro-intestinal branches were divided. Three to four months after recovery from the operation the effect of such a division of the vagi on the respiratory function of the lungs was tested in several ways and compared with similar experiments carried out on a dog with intact vagi. The following evidence was obtained.

1. The metabolism of the vagotomized dogs as evidenced by the oxygen consumption and the carbon dioxide elimination per minute, as well as the respiratory quotient, was in no way abnormal or demonstrably affected.

2. The respiratory rhythm in three out of the four dogs showed no abnormality whatever. One animal showed a very slightly prolonged wait at the end of expiration before beginning inspiration, together with a slight irregularity in respiration. This same dog immediately after operation exhibited a Cheyne-Stokes type of respiration, probably due to a post-operative partial pneumothorax.

3. The response of the respiratory mechanism to an increase of carbon dioxide and decrease of oxygen in the inspired air was in no way different from the normal response so well known in man or from that found in a normal animal.

4. The passage of oxygen, even at a low tension, through the lungs, whether by diffusion or by secretion, was not demonstrably affected by division of the pulmonary branches of the vagi.

CONCLUSION

The pulmonary branches of the vagus nerves do not appear to transmit impulses that control functions in any way essential to life. More specifically they do not appear to possess any demonstrable power over the normal regulation of the gaseous metabolism, the pulmonary ventilation, or the gas exchange in the lungs.

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TABLE III

Respiratory exchange

DATE	DOG	WEIGHT DOG	EXPERIMENT NO.	PREVIOUS MEAL		SPIROMETER EXPERIMENT			RESPIRATORY EXCHANGE					ACTIVITY OF DOG
				Time	Character	CO_2 per min.	O_2 per min.	Resp. Quo.	Carbon Dioxide		Oxygen		Resp. Quo.	
									per min.	per kilo per min.	per min.	per kilo per min.		
		kilos				*cc.*	*cc.*		*cc.*	*cc.*	*cc.*	*cc.*		
	Normal Dog													
Sept. 8	No. 1	5.6	1	9/7; 11 a.m.	Meat diet largely				29	5.2	41	7.3	0.70	Very quiet.
Sept. 8	No. 1	5.6	2	9/7; 11 a.m.	Meat diet largely				32	5.7	45	8.0	0.71	Very quiet
Sept. 8	No. 1	5.6	3	9/7; 11 a.m.	Meat diet largely				30	5.4	40	7.1	0.76	Very quiet
Sept. 9	No. 1	5.6	4	9/8; 3.30 p.m.	Meat diet largely				41	7.3	50	8.9	0.82	Shivered during entire experiment
									33	5.9	44	7.8	0.75	
	Vagus Dogs													
Aug. 24	No. 18	13.2	1	8/23; 12 n.	Meat diet largely	87	119	0.73	88	6.7	117	8.9	0.75	Very quiet
Aug. 24	No. 18	13.2	2	8/23; 12 n.	Meat diet largely	89	122	0.73	96	7.3	128	9.7	0.75	Occasional movements
Aug. 24	No. 18	13.2	3	8/23; 12 n.	Meat diet largely	80	116	0.69	94	7.1	124	9.4	0.76	Occasional movements
						93	134	0.69						
						87	123	0.71	93	7.0	123	9.3	0.75	

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TABLE III—Continued.

DATE	DOG	WEIGHT DOG	EXPERIMENT NO.	PREVIOUS MEAL		SPIROMETER EXPERIMENT			RESPIRATORY EXCHANGE					ACTIVITY OF DOG
				Time	Character	CO_2 per min.	O_2 per min.	Resp. Quo.	Carbon Dioxide		Oxygen		Resp. Quo.	
									per min.	per kilo per min.	per min.	per kilo per min.		
		kilos				cc.	cc.		cc.	cc.	cc.	cc.		
Aug. 27	No. 15	12.9	1	8/26; 4 p.m.	Meat diet largely	62	93	0.67	96	7.4	126	9.8	0.76	Whining during last three minutes of experiment
Aug. 27	No. 15	12.9	2	8/26; 4 p.m.	Meat diet largely	74	98	0.76	87	6.7	125	9.7	0.70	Restless
Aug. 27	No. 15	12.9	3	8/26; 4 p.m.	Meat diet largely	74	101	0.73	85	6.6	112	8.7	0.77	Very quiet
Aug. 27	No. 15	12.9	4	8/26; 4 p.m.	Meat diet largely				80	6.2	105	8.1	0.77	Absolutely quiet
Aug. 27	No. 15	12.9	5	8/26; 4 p.m.	Meat diet largely				96	7.4	127	9.8	0.76	Restless
Aug. 27	No. 15	12.9	6	8/26; 4 p.m.	Meat diet largely				88	6.8	116	9.0	0.76	Moved once
						70	97	0.72	89	6.9	119	9.2	0.75	
Sept. 2	No. 23	16.6	1	9/1; 3.30 p.m.	Meat diet largely				125	7.5	173	10.4	0.73	Very restless
Sept. 2	No. 23	16.6	2	9/1; 3.30 p.m.	Meat diet largely				96	5.8	136	8.2	0.71	Quiet
Sept. 2	No. 23	16.6	3	9/1; 3.30 p.m.	Meat diet largely				99	6.0	136	8.2	0.73	Occasionally restless
Sept. 2	No. 23	16.6	4	9/1; 3.30 p.m.	Meat diet largely				100	6.0	126	7.6	0.80	Very quiet
Sept. 3	No. 23	16.6	5	9/2; 3.30 p.m.	Meat diet largely				96	5.8	129	7.8	0.75	Very quiet
Sept. 3	No. 23	16.6	6	9/2; 3.30 p.m.	Meat diet largely				93	5.6	125	7.5	0.74	Moved once slightly
Sept. 3	No. 23	16.6	7	9/2; 3.30 p.m.	Meat diet largely				84	5.1	118	7.1	0.71	Absolutely quiet
									99	6.0	134	8.1	0.74	
Sept. 11	No. 11	14.2	1	9/10; 3.30 p.m.	Meat diet largely				122	8.6	137	9.6	0.89	Shivering continuously
Sept. 11	No. 11	14.2	2	9/10; 3.30 p.m.	Meat diet largely				87	6.1	107	7.5	0.81	Shivering part of experiment
Sept. 11	No. 11	14.2	3	9/10; 3.30 p.m.	Meat diet largely				82	5.8	102	7.2	0.80	Quiet
Sept. 11	No. 11	14.2	4	9/10; 3.30 p.m.	Meat diet largely				83	5.8	108	7.6	0.77	Shivering and somewhat restless
									94	6.6	114	8.0	0.82	

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DISTENSION OF THE LUNGS: ITS EFFECT ON THE RESPIRATION IN MAN AND IN NORMAL AND VAGOTOMIZED DOGS

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CLINIC OF PROFESSOR CUSHING

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Hering and Breuer[1] in 1868 first described an apnoea resulting from distension of the lungs. They produced the phenomenon in dogs by closing the trachea at the height of a normal or artificially increased inspiration. By the use of air containing little or no oxygen they further showed that the apnoea was not due to an excess of oxygen. They were unable, however, to obtain an apnoea by the same procedure after section of the vagi. They concluded, therefore, that the pause was a result of inspiratory inhibition due to the excitation of the vagi by stretching the lungs.

Their experimental results were duplicated by many observers and the phenomenon has become generally spoken of as the Hering-Breuer inhibitory effect.

Head[2] in 1889 reported from Hering's laboratory a very extensive study on the effect of distension and other forms of natural stimuli on the respiratory mechanism. At that time, however, it was not known that the respiratory centre was extremely sensitive to changes in the carbon dioxide tension of the arterial blood[3] and, therefore, many of the experimental results were

[1] Hering and Breuer: Die Selbsteuerung der Athmung durch den Nervus Vagus. Sitzgsber. d. Wiener Acad., Math.-natur., 1868, lvii, 672; lviii, 909.

[2] Head: On the regulation of respiration. Jour. Phys., 1889, x, 1–70; 279–290.

[3] Haldane and Priestly: The regulation of the lung ventilation. Jour. Physiol., 1905, xxxii, 225–266.

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erroneously interpreted as evidence of vagal influence. On the other hand, his experiments are very accurately described and profusely illustrated by kymographic records, and the data are still of great value and capable of reinterpretation.

Haldane and Lorrain Smith[4] later showed that, in rabbits, the pause in the respiration, produced during distension of the lungs, was not due to a lowering of the alveolar carbon dioxide pressure. In consequence, this particular experiment has survived as the only example of a "natural stimulus" which produces respiratory inhibition by stretching the vagi.

Very recently Christiansen and Haldane[5] have studied the effect of distension of the lungs on the human respiration. They made use of a bag containing air and so weighted that the air was under a pressure of 6 to 8 cm. of water. The subject, breathing through a mouthpiece, could be connected, by means of a three-way tap, either to the air of the room or to the air in the bag. They invariably found that distension of the lungs caused the respirations to cease, usually for about half a minute. The pause was then broken by a deep expiration, followed by a further pause before the next expiration, and so on with increasingly shorter pauses. On turning the tap, so that the subject again breathed atmospheric air, the pauses disappeared, and the breathing returned to normal. By the use of air containing 7.3 per cent carbon dioxide and 8.2 per cent oxygen, the pause is produced just as with pure air; the pauses succeeding the first, however, diminished in length much more rapidly than when pure air was used. This pause was, therefore, not dependent on a lowering of the carbon dioxide tension of the arterial blood.

In addition to the above Hering and Breuer "inhibitory" effect, which is not dependent on chemical changes in the blood, Christiansen and Haldane were able to obtain clear evidence of an independent phenomenon by the fact that under certain conditions an increase of carbon dioxide in the alveolar air will break

[4] Haldane and Lorrain Smith: The physiological effects of air vitiated by respiration. Jour. Path. and Bact., 1892, i, 168–186.

[5] Christiansen and Haldane: The influence of distension of the lungs on human respiration. Jour. Physiol., 1914, xlviii, 4, 272-277.

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through the inhibitory effect, and also that dilution of the alveolar carbon dioxide with pure air will augment the effect. They were able, therefore, to distinguish two distinct phenomena produced by distension of the lungs, the first being a so-called Hering-Breuer "inhibition," occurring with the thorax expanded, and the second being a true chemical apnoea, occurring with the thorax at the level of a normal expiration.

Throughout their paper, Christiansen and Haldane tactily accept, as an explanation of the primary pause, the Hering-Breuer theory of inspiratory inhibition from vagal excitation. That they are doubtful that this is the true explanation is evident from the following statement at the end of their paper: "Our experiments afford new confirmation to the view that true apnoea, apart from the inhibitory nervous effects produced during actual distension, or actual excitation along *certain other nerve paths* connected with the centre, is a 'chemical apnoea.'"

At the time of the appearance of Christiansen and Haldane's paper, investigations were being made in this laboratory on the influence of the vagi over the normal regulation of the gaseous metabolism, the pulmonary ventilation, and the gas exchange in the lungs.[6] As these studies failed to reveal evidence that the vagi possessed any demonstrable influence over these functions, we suspected that the apnoea following distension was likewise not due to vagal excitation.

For distending the lungs we made use of a Benedict respiration apparatus[7] and so weighted the spirometer that the air was under a pressure of 8 to 16 cm. of water. The subject sat in a chair and breathed through a wide-bore, three-way tap of which one branch led to the respiration apparatus, the second to the room air, and the third to the mouthpiece. The nose was closed with a clip. The subject at first breathed quietly for a short time

[6] Boothby and Shamoff: A study of the late effect of division of the pulmonary branches of the vagus nerve on the gaseous metabolism, gas exchange, and respiratory mechanism in dogs. This Journal, 1915, xxxvii.

[7] Benedict: Ein Universalrespirationsapparat. Deutschen Archiv. f. klinische Medizin. 1912, cvii, 157–200.

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through the branch of the tap leading to the room air, and at various periods in the respiratory cycle the tap was suddenly turned, so as to connect the subject with the air under pressure in the respiration apparatus. By this means the lungs were rapidly distended with air and the intra-pulmonary pressure increased by a definite amount. The spirometer of the respiration apparatus, though weighted, was perfectly free to move in either direction, so that the response of the subject to the stimulus of lung distension would be unhampered by still further changes in the intrapulmonary pressure, produced by any respiratory movements that might occur. In experiments in which the trachea is clamped or the tap entirely closed after the distension, changes in pressure would naturally follow any respiratory movements. Movements, therefore, that might be too small to be recorded by a pneumograph or even by a slip of the diaphragm could possibly produce changes in intrapulmonary pressure that would give rise to secondary and confusing reactions.

In the case of the dogs, an air-tight mask was applied to the animal's head, according to the method described by Boothby and Shamoff.[8] The mask was connected to the three-way tap by rubber tubing of large bore. The dead space of the mask and tubing was about 150 cc., which was quite large in comparison with the tidal volume of air. The dogs lay quietly on their sides on a table and were unrestrained. They were perfectly comfortable and did not require anaesthesia.

By means of a pneumograph around the thorax, the respiratory movements were recorded qualitatively on the kymograph, both before and after the subject breathed the air under pressure. As soon as the tap was turned to the respiration apparatus a very accurate quantitative curve of the coordinated total respiratory movements was written by the recording spirometer.

In Figure I is given a typical tracing of the effect, on W. M. B., of distending the lungs by suddenly increasing the intrapulmonary pressure. The tap is turned at the end of a normal

[8] Boothby and Shamoff: A study of the late effect of division of the pulmonary branches of the vagus nerve on the gaseous metabolism, gas exchange, and respiratory mechanism in dogs. This Journal, 1915, xxxvii.

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expiration, producing an exaggerated inspiration, as shown by the pneumograph curve below point *A*. The following expiration is abortive, as shown by both curves. The succeeding respirations are somewhat more rapid and slightly more irregular than when breathing under normal pressure. At *a* and *b* in this

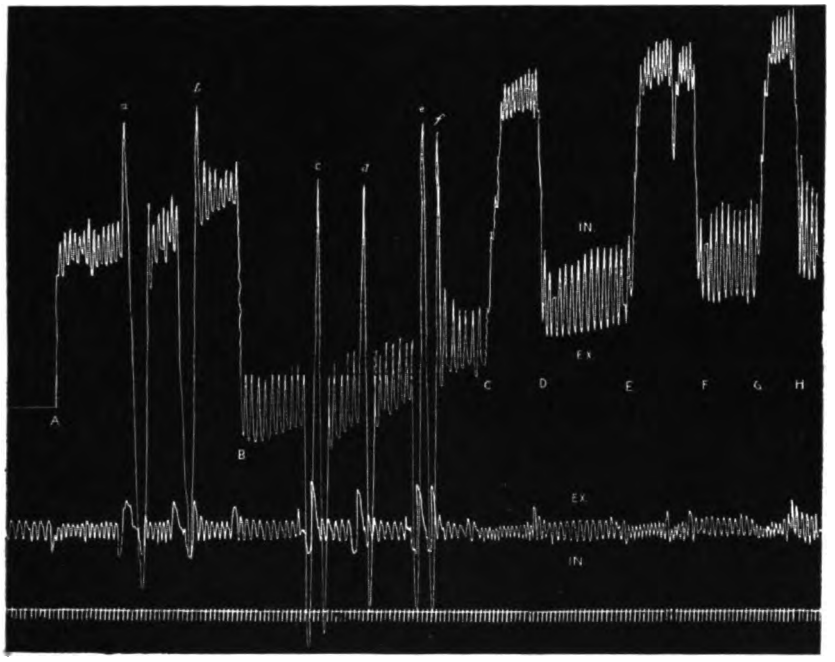


Fig. I. Effect of distension on W. M. B. Spirometer weighted to give 15 cm. water pressure. Upper curve written by the recording spirometer, with inspiration up and expiration down. Lower curve written by the pneumograph around the subject's chest with inspiration down and expiration up. To be read from left to right. At *A* tap turned so that the subject is connected with the air under pressure in the apparatus. The pneumograph shows qualitatively the preceding normal respiratory rhythm; the first effect is a total expansion of the chest, followed by faster and shallower respirations. At *a* and *b* maximum inspirations and expirations were made showing that the reserve air was greatly increased and the lungs markedly distended. At *B* the weights were removed from the spirometer and the subject then breathed air under a normal pressure. At *c*, *d*, *e*, and *f* maximum inspirations and expirations were made and are to be contrasted with those made at *a* and *b*. At *C*, *E*, and *G* the weights were replaced on the spirometer and at *D*, *F*, and *H* again removed. No evidence of apnoea either primary or secondary. Time in two seconds.

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figure maximum inspirations and expirations were made, showing that the "Mittelage" is much higher than under normal conditions. At *B* the weights were removed as quickly as possible, and at *c*, *d*, *e*, and *f*, maximum inspirations and expirations were again made to contrast the "Mittelage" under normal conditions with that obtained when breathing under pressure (*a* and *b*). At *C* the weights were reapplied, but, as they consisted of five lead weights, an appreciable time was occupied in putting them on the spirometer, thus producing a less sudden increase in intrapulmonary pressure than that obtained by turning the tap, as was done at *A*. The large drop in the spirometer at *A*, *C*, *E*, and *G*, and the corresponding rise at *B*, *D*, *F* and *H* are due only in small part to the change in the "Mittelage;" the greater part is caused by the compression (expansion) of the air to a smaller (larger) volume both in the apparatus and in the lungs by the addition (removal) of the weights on the spirometer.

Figure II shows another tracing for the same subject as in Figure I. Here again, we see no inspiratory inhibition. There is, however, in this curve an irregular pause of very short duration (about three seconds), beginning in complete inspiration. This pause, excepting for its irregularity, resembles those obtained by Christiansen and Haldane on distending the lungs. The notches on both the spirometer and pneumograph curves indicate that rhythmic expiratory movements were being made but were proving abortive.

This fact can be explained on the ground that the usual stimuli coming from the respiratory centre to the muscles of respiration were not sufficiently powerful to cause those muscles to completely contract against the increased load (pressure). After a few seconds the respirations again became practically normal, as by this time the respiratory centre was sending out stimuli of sufficient strength to cause the respiratory muscles to perform the increased work thrown upon them. In experiments following each other closely the delay before normal respiration was resumed markedly decreased, and, in fact, usually entirely disappeared, as is shown in the latter part of Figure II, where the distension effect was repeated without causing apnoea.

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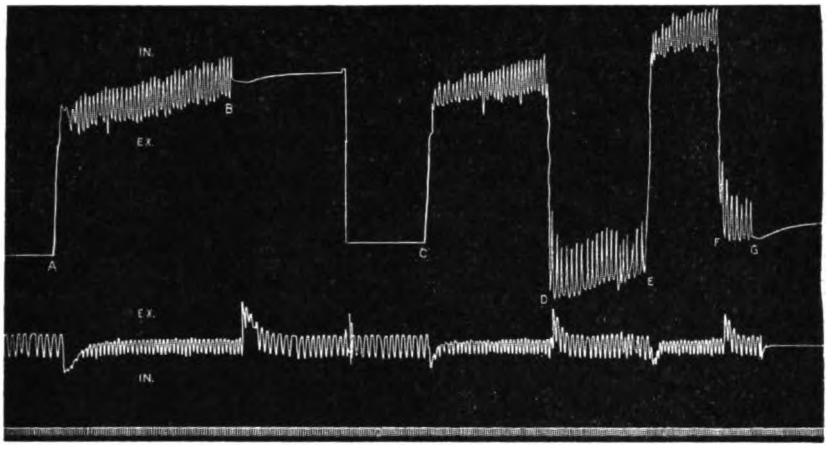


Fig. II. Effect of distension on W. M. B. Spirometer weighted to give 15 cm. water pressure. Upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. At *A* tap turned so that the subject breathed the air under pressure in the apparatus; this was followed by rhythmical but abortive attempts at respiration. At *B* and *G* tap turned to room air. At *C* tap turned to apparatus. At *D* and *F* weights removed from the spirometer and at *E* were reapplied. No apnoea at *C* and *E*. Time in seconds.

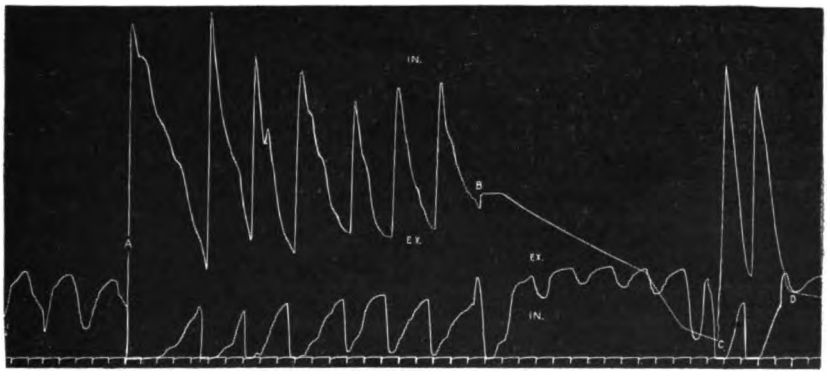


Fig. III. Effect of distension on F. B. B. Spirometer weighted to give 15 cm. water pressure. Upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. At *A* and *C* tap turned so that the subject breathed the air under pressure. At *B* and *D* tap turned to room air. The effect of distension is first to slow and deepen the respirations, then they become faster and shallower. Inspirations are very sharp. No apnoea. Time in seconds.

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The subject was careful to remain passive during every experiment and to refrain from any voluntary respiratory effort following the distension of the lungs. However, after repeated experiments, the subject appears to become somewhat accustomed to the unusual conditions and to unconsciously react more quickly to the distension. A similar adaptation occurs in the dogs, as we shall presently point out.

Figure III is a tracing obtained from another subject, F. B. B. The drum is revolving faster than in the previous figures and shows very clearly the irregularity of the first expiration. There is no delay in the commencement of the expiration but the several notches in the expiratory line indicate a somewhat irregular action or incoordination of the expiratory muscles. This curve illustrates very emphatically that there is no inhibition of inspiration in our subjects, as the next inspiration follows immediately after the completion of the expiration and is very sharp. In none of our curves nor in any of those given by Christiansen and Haldane is there any indication of a primary inspiratory inhibition. The distension apnoea in their curves is at the top of inspiration and is therefore an inhibition of expiration. The following chemical apnoea is at the bottom of expiration and shows a chemical inhibition of the respiratory movements. Figure IV is a reproduction of Figure 3 from Christiansen and Haldane's paper. Figures 5, 6, and 7 in their paper show conclusively that the secondary apnoea is chemical and not due to the same cause as the primary apnoea. The pause noted by Hering and Breuer is not at the top of inspiration but at the bottom of expiration, that is, the next expiratory phase is prolonged and inspiration is delayed, or, as they say, "inhibited" by

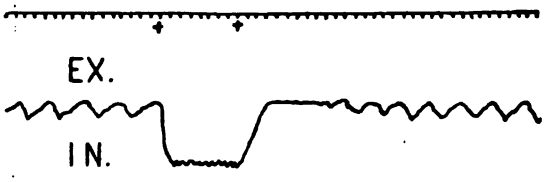


Fig. IV. Reproduction of Figure 3 from Christiansen and Haldane's paper (Jour. Physiol., xlviii, p. 274). "Effect of distension for 8 secs. Crosses show beginning and end of distension." Note the primary or distension apnoea in the position of deep inspiration and the secondary or chemical apnoea at the bottom of the normal expiration. Time in seconds.

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vagal stimulation. A typical example of the Hering-Breuer phenomenon is given in Curve VI, Plate II of Head's[9] excellent paper; we reproduce this curve in Figure V.

The curves of Christiansen and Haldane at first sight appear quite different from the typical Hering-Breuer phenomenon, as shown by Head's tracing in Figure V. In the former, the primary pause is at the top of inspiration, and in the latter, at the bottom of expiration. This discrepancy, however, can be accounted for by the way in which the curves are recorded. Christiansen and Haldane used a pneumograph around the chest wall, which roughly depicted the volume of the thorax, while Head made use of a slip of the diaphragm, which represents the condition of contraction or relaxation of that muscle. The volume of the lungs could be increased passively by the air pressure with the pause apparently in the inspiratory phase, as shown by Christiansen and Haldane, and yet the diaphragm would be relaxed, as shown by Head. In our experiments, in addition to the relaxation of the diaphragm, as occurs

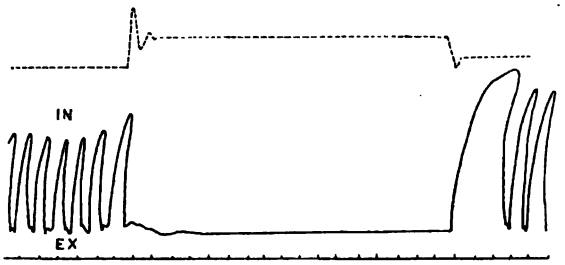


Fig. V. Reproduction of Curve VI, Plate II of Head's paper (Jour. Physiol., x). "The dotted tracing represents the movements of a mercury manometer connected with the trachea. Thus any rise on the curve represents a rise of pressure in the lungs after closure of the trachea. The respiratory curve is traced by the movements of the anterior slips of the diaphragm, separated and prepared according to the description in Head's paper, p. 4. Movement of the pointer up represents inspiration. From a rabbit weighing 1500 grams. Chloral 1¼ grams. The lungs were inflated during normal respiration and allowed to return to the normal volume before the pause had been broken by an 'interrupting' inspiration." On release of the pressure by opening the trachea, as shown by the dotted line, no movements of the diaphragm occurred until sufficient time had elapsed for the expiratory muscles to contract the thorax; then a big inspiration occured, as shown by the diaphragm reaction, followed by breathing which is deeper and slightly faster than before clamping the trachea. Time in seconds.

[9] Head: On the regulation of respiration. Jour. Phys., 1889, x, 1–70; 279–290.

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probably in the experiments of Christiansen and Haldane, as well as in those of Head, there is a coincident contraction of the inspiratory group of muscles, resulting in immediate expiration without producing any pause. As the trachea was clamped in the case of Head's experiments, the air could not be expired even if the expiratory muscles contracted, and any attempt to do so would increase materially the distension effect.

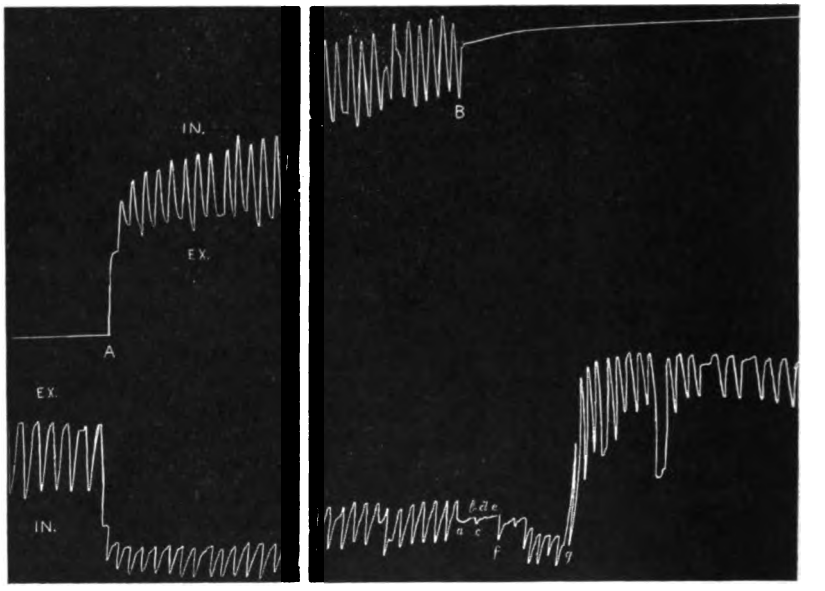


Fig. VI. Effect of distension on W. M. B. Spirometer weighted to give 15 cm. water pressure. Upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. At *A* tap turned to apparatus. At *B* tap turned at the top of inspiration so that the air-way was entirely closed, producing the effect of clamping the trachea. The pneumograph curve shows essentially a pause lasting a considerable time; the pause, however, is broken by the notches *a*, *b*, *c*, *d*, *e*, and *f*, and finally by regular respiratory movements, although no air could go in or out of the lungs. At *g* tap was turned so that the subject could breathe normally the room air.

Figure VI is another tracing from W. M. B. At *A* the tap was suddenly turned so that the subject breathed the air under pressure in the respiration apparatus. At *B* a special tap on the

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mouthpiece was closed near the end of inspiration in such a manner that the subject's air-way was entirely closed. This procedure is equivalent to clamping the trachea with the lungs distended. From the pneumograph curve, it is evident that the inspiratory movement almost instantly stopped, though probably not completely, as shown by the slight notch at *a*. The chart level then fell slightly at *b* but at *c* there is a sharp inspiratory movement. This inspiration was abortive, as no air could enter the thorax, and immediately a negative pressure was probably produced. The inspiration at *c* is slightly delayed, but the following notches *d* and *e* are very close to the normal respiratory rhythm. At *f* the subject makes a more pronounced inspiratory effort, followed by one less marked and then five which are very pronounced. The tap is then opened at *g*, so that the subject can breathe room air. The notches indicating inspiratory attempts do not show in all our tracings, though by observing our own reactions we feel sure that they occur. Their absence in some of the experiments may be explained by the fact that the rhythmic stimuli are not strong enough to cause the respiratory muscles to contract sufficiently to produce a change in the shape of the thoracic wall, so that a record would be made by the pneumograph. Apparently, the absence of the notches is not due to complete cessation of the respiratory stimuli.

In the distension experiments on ourselves we did not obtain any indication of the secondary or chemical apnoea, so well shown by Christiansen and Haldane. The failure of such an apnoea to occur in our subjects is, however, to be expected, as in neither of them is the most violent and prolonged forced breathing followed by apnoea.[10]

Figure VII is a tracing obtained from a normal dog. The tap is turned at various points in the respiratory cycle, so that the animal first breathed the air of the room, and then the air under pressure in the respiration apparatus. The upper curve is that written by the spirometer and the straight lines are made when the dog is breathing the room air. The lower curve is that ob-

[10] Boothby: Absence of apnoea after forced breathing. 1912, Jour. Physiol, xlv, 5, 328–337.

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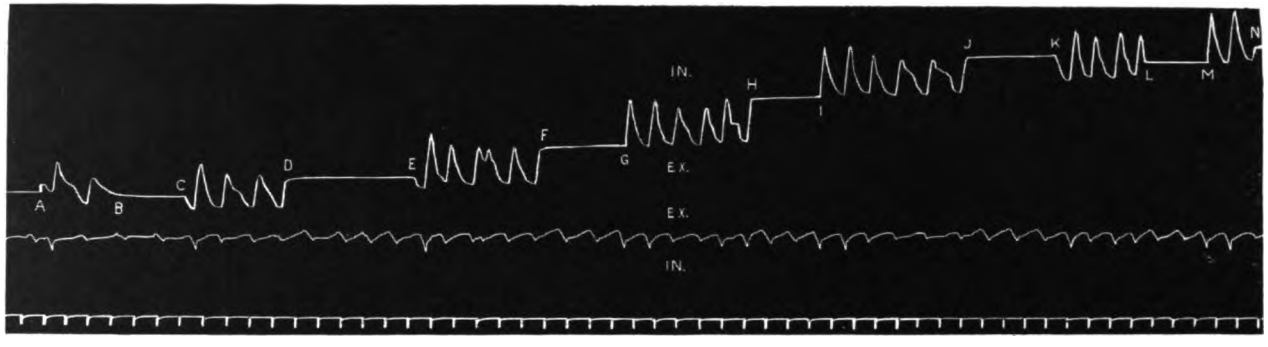


Fig. VII. Effect of distension on normal dog No. 1. Spirometer weighted to give 10 cm. water pressure. Upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. At *A*, *C*, *E*, *G*, *I*, *K*, and *M* the tap was turned at various phases of the respiratory cycle, so that the dog breathed the air under pressure in the apparatus. At *B*, *D*, and etc., the tap was turned so that the dog breathed the room air, and the following horizontal lines do not indicate periods of apnoea, but that the animal is not at that time connected to the apparatus. No apnoea. Time in seconds.

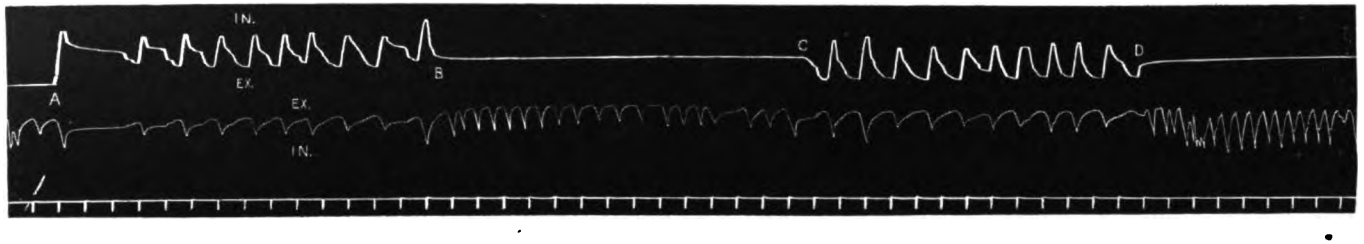


Fig. VIII. Effect of distension on normal dog No. 1. Spirometer weighted to give 12 cm. water pressure. At *A* the tap turned so that the dog breathed the air under pressure. This was followed by an abortive expiration with a subsequent pause. At *B* the tap was turned to the room air. At *C* tap again turned to respiration apparatus. No apnoea. Time in seconds.

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tained by means of the pneumograph. It does not show, as do those obtained from the human subjects, the change in the "Mittelage," because the length of the pneumograph necessitated its being passed over the chest of the dog and under the table on which the dog was lying. No evidence of inspiratory inhibition is seen here nor any sign of the primary distension apnoea shown by Christiansen and Haldane, and therefore resembles the curves obtained on ourselves.

Out of a long series of experiments on this same animal, only one presented a trace of expiratory difficulty, as shown in Figure VIII. It was the first experiment performed on this animal and two seconds elapsed before the dog was able to complete the expiration. After that the animal was ready to overcome the distension immediately, as did the human subjects mentioned above.

In the normal dog we were unable to obtain any example of the delayed or chemical apnoea following distension of the lungs.

In order to study the effect of increased intrapulmonary pressure with distension of the lungs, after division of the vagi, we used two dogs in which we had some three to four months previously divided the pulmonary branches of both vagi.

The results obtained from the dogs with the vagi divided in this manner were quite surprising. In one animal (No. 23), two out of seven experiments showed a primary apnoea on distension; one lasted six, and the other two and one-half seconds. Sixteen experiments were performed on the other dog, (No. 11); in nine of which there was no apnoea; in one an apnoea of one and one-half seconds; in three an apnoea of about three seconds, in one an apnoea of four and one-half seconds, and in two an apnoea of four and one-half seconds, occurring after several respirations.

In Figure IX is shown the longest primary apnoea obtained. The tap was turned so that the animal was suddenly made to breathe the compressed air. This was accompanied by dilatation of the thorax, as shown by the lower (pneumograph) curve, followed immediately by an incompleted expiration. The expiratory movement, however, does not expel any air as shown by the spirometer curve. The thorax remains stationary for

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six seconds and the apnoeic period is finally broken by an inspiration. The next two respirations are somewhat slowed by a prolongation of the expiratory phase, after which the rhythm becomes quite normal.

Another example is given in Figure X, and a third in the first part of Figure XI in which the apnoeic period was broken by inspiration with distinct prolongation of the expiratory phase. It

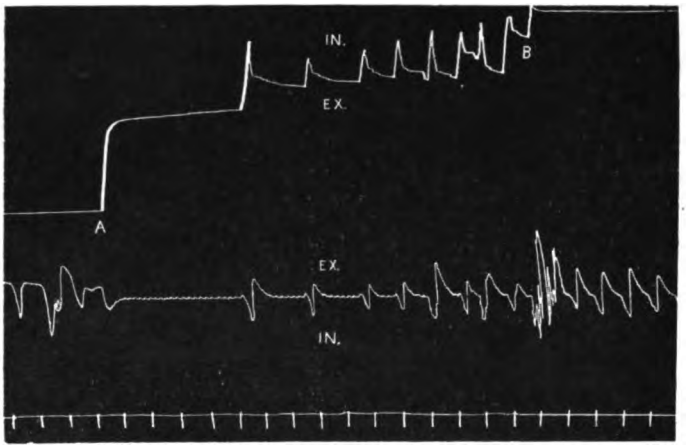


Fig. IX. Effect of distension on vagotomized dog No. 23. Spirometer weighted to give 12 cm. water pressure. Upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. At *A* tap turned so that the animal breathed the air under pressure, causing an inspiration, followed by a partial expiration as shown by the pneumograph curve. After this there was a pause of six seconds broken by an inspiration. The pause was at the level of the following expirations. The subsequent respirations were slightly slowed and the expiratory phase was prolonged. Time in seconds.

is questionable whether the prolonged expiratory pause at *D* in Figure XI should be considered an example of the chemical apnoea of Christiansen and Haldane, or whether it is an exaggerated case of the prolonged expiratory phase seen in the first part of this figure and in Figures IX and X. On account of the relatively large dead space of the mask and connecting tubes, it seems unlikely that distension of the lungs would materially lower the alveolar carbon dioxide tension. We believe, therefore,

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that this pause is similar to the other, though shorter, examples of a prolonged expiratory phase found in our vagotomized dogs and not an instance of a true chemical apnoea.

Our experiments on man supplement those of Christiansen and Haldane and together they show that a primary apnoea may or may not occur in normal individuals upon distension of the lungs.

Our experiments on two dogs in which the pulmonary branches of both vagi were divided and in which we obtained several instances of a short apnoea on distension of the lungs is direct evidence against the theory that the vagi transmit impulses which arise from distension of the lungs and thereby inhibit inspiration. Our curves on the vagotomized dogs are all the more suggestive because the apnoea, when it does occur, is at the end of the following partial or complete expiration which is broken by an inspiration. They suggest quite strongly that there is a condition of inspiratory inhibition. If so, it can not be due to impulses arising in the lungs and passing over the vagi, as the pulmonary branches are all divided.

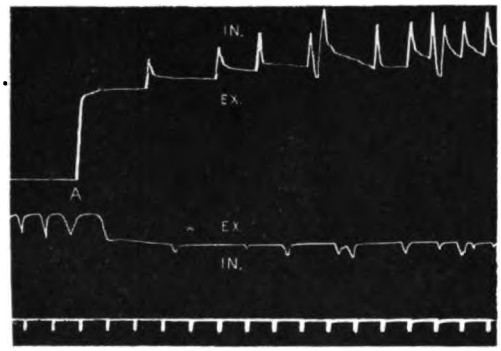


Fig. X. Effect of distension on vagotomized dog No. 23. Spirometer weighted to give 12 cm. water pressure. Upper curve written by spirometer, with inspiration up. Lower curve written by pneumograph, with inspiration down. The upper pointer is writing to the left of the lower pointer. At *A* the tap turned so that the animal breathed the air under pressure, causing an inspiration, followed by a pause of two and one-half seconds, broken by an inspiration. The next respiration shows a prolonged expiratory phase, as do the following to a lesser degree. Time in seconds.

In another paper from this laboratory by Boothby and Shamoff,[11]

[11] Boothby and Shamoff: A study of the late effect of division of the pulmonary branches of the vagus nerve on the gaseous metabolism, gas exchange, and respiratory mechanism in dogs. This Journal, 1915, xxxvii.

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other experiments are reported which show that the pulmonary branches of the vagus nerve do not transmit impulses that control functions in any way essential to life. And more specifically the nerve does not possess any demonstrable power over the normal regulation of the gaseous metabolism, the pulmonary ventilation, or the gas exchange in the lungs.

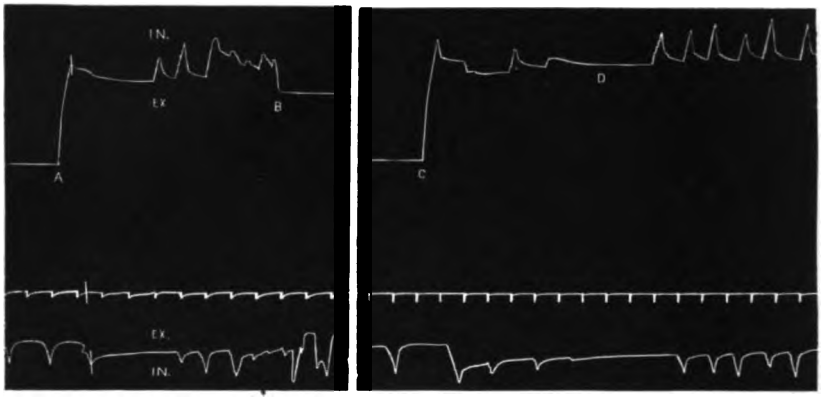


Fig. XI. Effect of distension on vagotomized dog No. 11. Spirometer weighted to give 12 cm. water pressure. The upper curve written by the spirometer, with inspiration up. Lower curve written by the pneumograph, with inspiration down. Upper pointer is writing to the left of the lower pointer. At *A* and *C* tap turned so that the animal breathed the air under pressure. At *A* the first effect of distension is expansion of the chest, followed by a slight contraction after which there is a pause of about three seconds broken by an inspiration. The pause is on a level with the bottom of the following expirations. At *C* the inspiration following the distension is more marked and is again repeated in about a second followed by a pause of about two seconds. This is broken by an inspiration followed by a prolongation of the expiratory phase. The next expiratory phase is very much prolonged (4 secs.). The respirations then become quite normal. Time in seconds.

The experiments reported in this paper indicate that the nerve does not transmit impulses which, according to the theory of Hering and Breuer, arise from distension of the lungs and inhibit inspiration.

Respiration is the result of a highly coordinated response of many muscles to the need of the body for a constant exchange of gases to and from the air. It is normally an involuntary and

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rhythmic act under the control of what has been designated as the respiratory centre and what we prefer to call the ventilation centre. Respiratory rhythm, unlike cardiac rhythm, must be easily and instantaneously modified to meet the needs of an entirely different order, such as phonation, delugition, and the application of external force to the thorax. Therefore the rhythmic stimuli sent out by the ventilation centre must be weak, so that stimuli from any other source may instantly halt or change the respiratory movement at any stage of the cycle.

In different individuals the strength of the rhythmic stimuli sent out by the centre may readily vary. Certain other stimuli may affect the respiratory rhythm in one person with greater rapidity and in quite a different way from that in another person.

Inspiration under usual conditions is a muscular act and consists in distending the thorax with a disturbance of elastic equilibrium. Expiration, on the other hand, when the body is at complete rest, is largely a passive act and is the return of the thorax to its former position of elastic equilibrium.

Under many circumstances, however, such as work, phonation, breathing against an obstructed air-way or against positive pressure, expiration is no longer passive but active and must, therefore, be performed by the contraction of the expiratory group of muscles. Inspiration may then become the passive act.

In the human subjects and in the normal dog studied by us, the expiratory group of muscles were almost always in tone and ready for work, and the rhythmic expiratory stimulus was of sufficient strength to cause them to contract normally in spite of an additional and instantaneous overload. On the other hand, with the subjects studied by Christiansen and Haldane, some thirty seconds elapsed before the centre sent out stimuli sufficiently strong to cause the expiratory muscles to contract against the sudden overload. That is, in those subjects the time reaction was slow. In other words, it took thirty seconds for the ventilation centre to become adjusted, probably through the coordinating action of some higher centre, so that stimuli of sufficient strength would be sent out to cause muscular contraction of the expiratory muscles.

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In the dogs with divided vagi the expiratory muscles started to contract the moment the inspiration was completed. This contraction is seen distinctly in the pneumograph curve in Figure IX but is only slightly indicated in Figure X. The expiratory impulses sent out by the centre were strong enough to keep the thorax contracted to a certain level but could not carry it further. In this instance the failure to expire completely does not seem to be due to lack of proper stimuli but to actual inability of the muscles to contract more against the increased resistance, because the later expirations do not reach a lower level. In these experiments the respiratory centre was sending out stimuli that kept the expiratory muscles contracted to a certain level. The stimuli or the muscles themselves were, however, unable to complete the expiratory act. It is very probable that the apnoea lasted until the automatic ventilation centre received impulses from some higher coordinating centre so that the action of the ventilation centre was reversed and stimuli sent out to the inspiratory group of muscles.

In the experiments performed by us on the dogs without any pulmonary vagal supply, these nerves had been divided several months previously, so that the animals had had time to readjust themselves to the loss of vagal activity. It is well known, as has been shown by Gad[12] and others, that immediately following freezing or dividing the vagi there is a distinct change in the respiratory rhythm and the form of the respiratory curve. After division of one vagus, the return to normal takes place usually within one or two minutes. Lewandowsky[13] shows tracings taken five and again twenty-four hours after division of both vagi in the neck; in the first, there is still a distinct variation from the normal, but in the second, the character of the respiratory curve has returned to normal, though it is still a trifle deeper and slightly slower.

[12] Gad: Die Regulirung der normalen Athmung. Arch. f. Physiol., 1880, 1–30.

[13] Lewandowsky: Die Regulirung der Athmung. Arch. f. Physiol., 1896, 4, 196-248; 483-510. Tracings 64, 65, 66.

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The vagus nerve undoubtedly has some important function in respiration and the interruption of that function produces temporary changes in the respiratory rhythm. This may readily be conceived to occur even if its normal function is in no way concerned with transmitting impulses that arise from dilatation or collapse of the lungs. It may well be, as maintained by Krogh,[14] that the nerve has in the lungs a vasomotor function; or, as suggested by Brodie and Dixon,[15] it may be concerned with the dilatation and constriction of the bronchioles; or it is not unlikely that it might be concerned with both these functions.

The cause of the primitive rhythmic activity of the ventilation centre is unknown. It is recognized, however, that the rhythm and the volume of the respiration are influenced by many factors, the chief of which is the hydrogen ion concentration of the blood passing through the ventilation centre. The secondary factors influencing the centre are stimuli which enter the central nervous system over nearly every centripetal nerve[16] in the body and which are probably modified by a most complex and practically unknown coordinating mechanism.

SUMMARY

In this paper we have studied the effect of distension of the lungs on the respiratory rhythm in man and in normal and vagotomized dogs. We were unable to obtain any evidence to substantiate the theory suggested by Hering and Breuer that distension inhibited inspiration by the stimulation of the intrapulmonary endings of the vagus nerve.

[14] Krogh: On the mechanism of the gas exchange in the lungs of the tortoise. Skand. Arch. f. Physiol., 1909, xxiii, 200–216.

[15] Brodie and Dixon: The bronchial muscles, their innervation, and the action of drugs upon them. Jour. Physiol., 1903, xxix, 97–173.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

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2. Each article should conclude with a brief summary of results, suited to the needs of reference journals.

3. Reference to literature cited should contain, first, the name of the author or authors; second, the name of the journal or book; third, the year; fourth, the volume number and fifth, the page. Thus, Meltzer: American Journal of Physiology, 1908, xxi, 449. References should be numbered serially and be gathered at the end of the paper.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 37 JUNE 1, 1915 No. 3

THE ACTIVE PRINCIPLES OF DIFFERENT ORGANS, AS SHOWN IN KYMOGRAPH TRACINGS[1]

GEORGE G. FAWCETT, JOHN ROGERS, JESSIE M. RAHE AND S. P. BEEBE

Received for publication April 1, 1915

A recent report from the Department of Experimental Therapeutics in the Cornell University Medical College described the effects produced by the intravenous injection in dogs of certain proteids and extractive materials which can be isolated from an aqueous extract of the thyroid gland.[2]

The nucleoproteins, globulins and coagulable proteins are each found to be entirely without influence upon the kymograph tracings except in massive or overwhelming dosage. This was contrary to what one of us had expected because when the nucleoproteins and globulins are administered to patients who present symptoms of thyroid disease, these substances seem very active in either relieving or intensifying the preëxisting disturbance.

After the removal of the nucleoproteins, globulins and coagulable proteins, the filtrate which remains was found to produce a marked fall in blood pressure, a slight acceleration of the pulse rate, and a slight deepening of respiration. This filtrate was

[1] Report from the Johnston-Livingston Fund for Experimental Therapeutics in the Department of Experimental Therapeutics, Cornell University Medical College.

[2] American Jour. of Physiology, vol. xxxvi, no. 2, Jan. 1915, p. 113.

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then evaporated to dryness and extracted with alcohol, and then only the alcohol soluble portion proved to contain the active material. This was then further subdivided into a lead precipitate and a lead filtrate; the latter liquid alone proved active in the same way and in the same proportion as the original first filtrate from which it was derived. Therefore, the first filtrate, which can be prepared with comparative ease and cheapness and was found to be sterilizable without altering its properties, has been employed for all subsequent experiments under the designation of the thyroid "residue." It signifies the portion of an aqueous extract of the thryoid which remains after the removal of the nucleoproteins, globulins and coagulable proteins. All the substances which can be separated from the thyroid by the means we have employed contain iodin of which the largest amount is found in the nucleoproteins.

As is well known, more iodin is present in the pig than in the sheep thyroid. When similar amounts of the pig and of the sheep "residues," calculated according to their nitrogen content, are injected intravenously, the pig thyroid residue produced a greater fall in blood pressure than the sheep "residue." When, however, the dosage, calculated according to the iodin content, is the same, the resultant fall in blood pressure is precisely the same. Hence the active vasodilating principle of the thyroid "residue" apparently contains iodin.

After studying the effects of the different materials which can be recovered from an aqueous extract of the thyroid, it has seemed advisable to compare the results with a similar series of experiments with the materials obtained in a corresponding manner from other organs. It was found that the nucleoproteins, globulins and coagulable proteins recovered from aqueous extracts of the thymus, pancreas and liver, like those from the thyroid, were entirely inert, while the filtrates or "residues," as they will hereafter be called, which remained after the removal of these bodies were uniformly active. Therefore, in the subsequent tests only the "residue" of organ extracts have been employed. Contrary to the experience of others who had used somewhat similar materials, we have not noted the estab-

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lishment of any perceptible tolerance to these "residues" after their repeated injection.

All the "residues," or first filtrates, like that of the thyroid have shown a more or less marked depressor effect, except the "residue" prepared from the adrenal gland. This power of producing fall in blood pressure, possessed by watery extracts of so many organs has long been known and has generally been believed to be caused by a common cholin content. We have, therefore, prepared cholin by Hoppe-Seyler's method, and have found that an amount of cholin which is comparable in its nitrogen content to a thyroid "residue," for example, produces in the kymograph tracings no fall in blood pressure. Hence, the vasodilating principle is not cholin. Vincent[3] and others have demonstrated that cholin cannot be present in at least some of the extracts and, therefore, the depressor effect must be due to some other substance. This may be the same in every organ and be represented by what Popielski[4] designates as "vasodilatin;" or it may be a material peculiar to and characteristic of each organ. Popielski worked with the same organs as we have, but the material he isolated and used for his experiments differs much in its character and methods of preparation from our material. After a comparison of all the observations, however, it is more reasonable to believe in a common vasodilating principle than in a number of different principles having the same effect. It seems probable that a substance, some part or all of which has a vasodilating effect, exists in, or is produced by, all organs in amounts which vary in each. For in our experiments the "residue," or portion of the extract of each organ which is demonstrably active in the kymograph tracings, produces a fall in blood pressure which is more or less characteristic for the "residue" of the particular organ employed when standardized according to its nitrogen content and compared with a similar amount of thyroid "residue." The other differences which can be detected in the secondary rise after the primary fall in blood pressure (iv, pancreas residue, v, pituitary, viii, thymus), and

[3] Vincent: Int. Secretions and Ductless Glands, 1912, p. 31.

[4] Popielski: Chemical Abstracts, vol. 7, no. 4, Feb. 20, 1913, p. 613.

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the alteration in the depth of respiration (i, thyroid, iii, liver, v, pituitary, vii, spleen), when compared with the characteristic fall in blood pressure, produced by similar amounts of each "residue," standardized according to its nitrogen content suggest that every organ is capable of producing some particular material which is active in the sense of having some specific effect. It is also possible that this material consists of a combination of some depressor substance common to all organs, with another body which constitutes a secondary and different active principle in every organ.

As it might be possible that the depressor agents in the "residues" were due to concentrated salts or to some form of protein which had escaped removal and so might be common to all "residues," we have tested the effects of intravenous injections of salts and proteins in varying dosage, but always with negative results. The preparations employed consist of sodium iodid, potassium iodid, calcium iodid, calcium citrate, sodium citrate, sodium chloride and Witte's peptone. In doses of 5 cc. to 10 cc. of a 2 per cent solution these substances do give a vasodilatation effect, but these amounts are so far in excess of any possible content in our "residues" that they cannot be taken into consideration. The "residue" from the adrenal gland has been found to contain epinephrin in a concentration of approximately 1 to 250, as compared to the 1:1000 solution of adrenalin chloride prepared by Parke, Davis & Co., and is the only "residue" which shows a pressor effect. That from the pituitary gland, standardized and compared with the others according to the nitrogen content, shows the most marked primary depressor effect which is followed later by a rise in blood pressure.

Both of these glands are peculiar in the different structure and different physiological action of their anatomically closely related parts, and its does not seem probable that either organ normally pours into the circulation two disassociated secretions. It is more reasonable to believe that the pressor principle, or the similar "pituitrin" and epinephrin exist during life in combination with some less easily demonstrable or secondary active principle which comes from the other half of each gland. If this is

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true, the "residue" of these glands which correspond to all other "residues," should indicate it, and that of the pituitary which produces the greatest fall in blood pressure of all the "residues," and is followed by a secondary rise with marked deepening of respiration, is certainly suggestive. The adrenal "residue" is the only "residue" which produces a primary pressor effect, but its tracings differ materially from those of adrenalin.

The experiments have been made with the following extracts, standardized according to their protein content:

Residue	*Strength of solution in mgs. of protein in cc.*
Sheep thyroid.....	30.0 mgs. of nitrogen calculated as protein per cc. of solution
Pig thyroid.....	26.6 mgs. of nitrogen calculated as protein per cc. of solution
Parathyroid.....	16.0 mgs. of nitrogen calculated as protein per cc. of solution
Liver.....	170.0 mgs. of nitrogen calculated as protein per cc. of solution
Pancreas.....	157.5 mgs. of nitrogen calculated as protein per cc. of solution
Pituitary.....	16.0 mgs. of nitrogen calculated as protein per cc. of solution
Muscle.....	35.0 mgs. of nitrogen calculated as protein per cc. of solution
Spleen.....	20.0 mgs. of nitrogen calculated as protein per cc. of solution
Thymus.....	64.0 mgs. of nitrogen calculated as protein per cc. of solution
Adrenal.....	18.9 mgs. of nitrogen calculated as protein per cc. of solution
Cholin.....	6.0 mgs. of nitrogen calculated as protein per cc. of solution

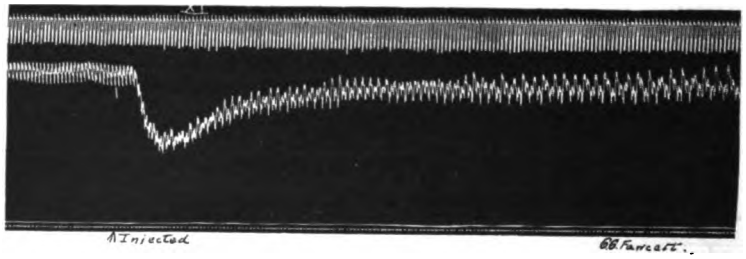
When the "residues" are made under closely similar conditions, that is when the duration of the period in which the extract is made, the temperature at which it is conducted and the proportion of water to hashed gland and the degree of freshness of the organ, are all about the same for each gland extract, its content of nitrogen is quite characteristic of the organ from which that "residue" has been obtained. When a dose of "residue" from any organ is so calculated as to contain the same amount of nitrogen as the dose of thyroid "residue," the fall in blood pressure which it produces, when injected intravenously in a dog, is different for each of the organs tested. The resultant curves in the kymograph tracings, when the dose of the nitrogen content of each "residue" is kept the same, show distinct differences which seem peculiar to and characteristic of the organ from which the "residue" has been derived.

A summary of these results is given in the following table. The figures were all obtained from the same dog, as there are slight variations in different animals.

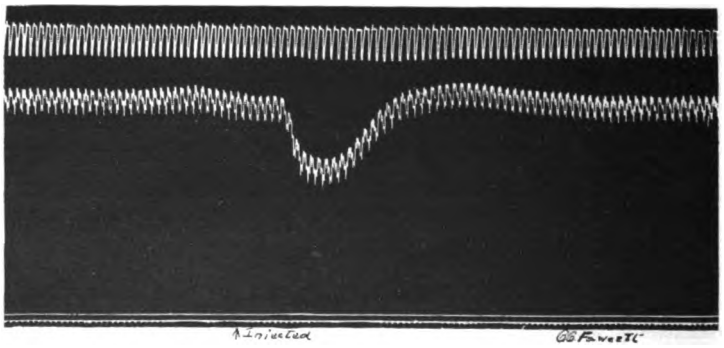
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Residue	*Fall in blood pressure in mms. of Hg.*	*Mgm. of nitrogen calculated as protein*
Pituitary	69	26.6
Liver	58	26.6
Thyroid	52	26.6
Spleen	50	26.6
Thymus	48	26.6
Pancreas	40	26.6
Parathyroid	36	26.6
Muscle	32	26.6

The results of intravenous injections of these "residues" in quantities which contain the same amount of nitrogen are shown in the following tracings, numbered and labeled with the table to explain each. All the tracings are taken from one dog, with tracing XI as the check on the series.

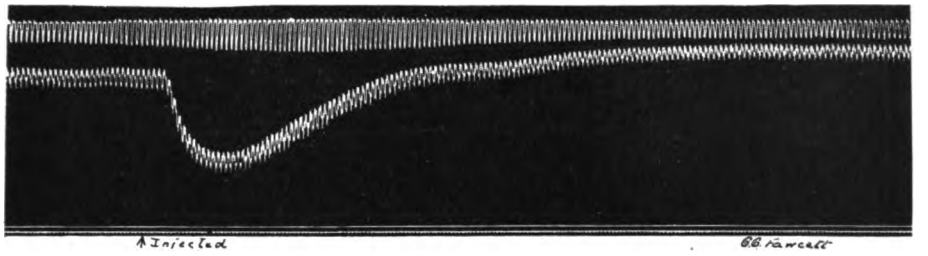


No. III. Liver residue. For dosage see table.

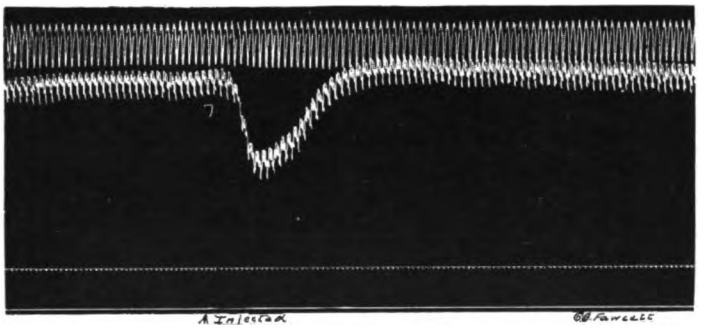


No. IV. Pancreas residue. For dosage see table.

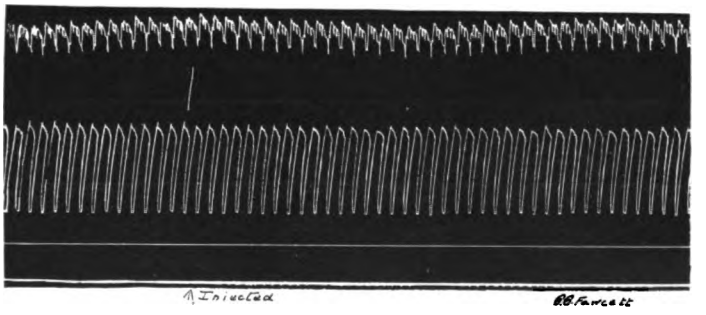
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No. V. Pituitary residue. For dosage see table.



No. VIII. Thymus residue. For dosage see table.



No. XVI. Cholin. For dosage see table.

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TRACING NUMBER		I	II	III	IV	V	VI	VII	VIII	IX	X	XI NOTE I	XII NOTE II	XIII	XIV NOTE III	XV NOTE IV	XVI NOTE V
Extract used		Thyroid residue "pig"	Para-thyroid residue	Liver residue	Pancreas residue	Pituitary residue	Muscle residue	Spleen residue	Thymus residue	Thymus globulin	Thymus albumin	Thyroid residue "pig"	Thyroid residue "pig"	Cholin	Thyroid residue "sheep"	Thyroid residue "sheep"	Cholin
Strength of Solution in N. Calculated as Protein Mgs. per cc.		26.6	16.	170.	157.5	16.	35.	20.	64.			26.6	17.5	16.	30.	30.	6.
Dosage	Minims	16.	26.6	2.5	2.7	26.6	12.12	21.2	6.6			16.	5.	15.	3.	8.	32.
	Mg's Protein	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26 6	26 6	5.1	5.1	5.1	15.	12.
Effects on Blood Pressure	Fall in Mm's. Hg	52.	36.	58.	40	60.	32.	50.	48	0	0	50.	32.	0	22.	30.	0
	Rise in Mm's. Hg	0	0.	0	Second-ary 6 mms.	Second-ary 20 mms.	0	0	Second-ary 6 mms.	0	0	0	0	0	Second 4 mms.	0	0
	Time before Effect	6 sec.	4 sec.	4 sec.	6 sec.	6 sec.	7 sec.	6 sec.	4 sec.	0	0	3 sec.	3 sec.	0	4 sec.	4 sec.	0
	Time of Action	2 min 5 sec.	3 min.	3 min. 20 sec.	1 min.	5 min.	35 sec.	1 min. 10 sec.	2 min.	0	0	2 min. 6 sec.	1 min. 30 sec.	0	44 sec.	44 sec.	0
Effects on Pulse Rate	Normal Rate per Minute	186	180	180	180	180	180	180	180	180	180	180	198	180	180	180	180
	Rate at Lowest Point of Bl'd. P.	183	176	198	180	168	180	180	176	0	0	180	194	180	180	180	184
	Rate during return of Bl'd. P. to Normal	186	180	198	180	174	180	180	174	0	0	180	185	180	184	180	184
	Rate after Bl'd. P. returns to normal	186	180	186	180	168	180	180	176	180	180	180	190	180	180	180	184
Effects on Respiration	Rate of Resp. at different Pts. of Bl'd. P. — Normal per Min.	86	72	60	48	42	48	48	45	45	48	48	48	48	45	45	41
	Lowest point	86	72	60	44	42	48	48	45	45	48	48	48	46	48	45	41
	Returning to Normal.	86	70	58	44	44	48	48	45	45	48	52	46	46	47	45	41
	Normal	86	66	58	46	46	48	48	45	45	48	48	48	48	47	43	41
	Amplitude of Resp. at different Pts. of Bl'd P. — Normal		No Change	No Change	No Change	Normal Increase	No Change	—	No Change	No Change	No Change	Slight Increase	No Change	No Change	No Change	No Change	No Change
	Lowest point	Slight Increase				Increase		—									
	Returning to normal.							Very Slight Increase									
	Normal					Decrease											

Notes. I. Thyroid residue was given here to check the condition of the dog's blood pressure.—The results are practically identical with Tracing No. I.

II. New preparation of thyroid "Residue" from pigs' thyroids. Iodin = 0.02 mgs. per cc.

III. Residue from sheeps' thyroids—Protein dosage same as Tracing No. XII.

IV. Iodin content of sheep-thyroid residue = 0.01143 mgs. per cc. Dosage of this made to correspond to iodin dosage of pig-thyroid residue of Tracing No. XII.
Iodin dosage of Tracing No. XII = .0062 mgs.
Iodin dosage of Tracing No. XV = .0062 mgs.

V. Dosage of Cholin = 12 mgs. which is more than twice the protein dosage of the thyroid residues of Tracings Nos. XII and XIV.

G. G. Fawcett.

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CONCLUSIONS

(The term "residue" is used to designate that portion of an aqueous extract of an organ which remains after the removal of the nucleoproteins, globulins and coagulable proteins.)

1. The "residue" is the only part of an aqueous extract of the thyroid, thymus, pancreas, and liver which contains demonstrably active depressor agents, and it is, therefore, presumably true of all organs.

2. All the different organs from which we have prepared extracts, with the exception of the adrenal, contain a depressor substance within the "residue" portion, which in each case varies in its action directly as its nitrogen content (calculated in terms of protein).

3. The depressor effect of the thyroid "residue" is dependent upon a substance containing iodine.

4. When standardized according to its nitrogen content each "residue" produces characteristically different effects in the kymograph tracings.

5. This depressor agent cannot be cholin because a dosage of pure cholin which is comparable to that of a thyroid "residue" for example, produces no effect.

6. The active portion of these "residues" is apparently not the same as Popielski's vasodilatin, which is prepared in a totally different way and gives different reactions.

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THE RATE OF OXIDATION OF ENZYMES AND THEIR CORRESPONDING PRO-ENZYMES

W. E. AND E. L. BURGE

Physiological Laboratory of the University of Illinois

Received for publication April 1, 1915

This investigation was begun primarily to determine if there is any difference in the ease with which trypsin and trypsinogen, pepsin and pepsinogen can be oxidized.

TRYPSIN AND TRYPSINOGEN

Pancreatic juice was collected from dogs according to the ordinary method. One grain of morphia was injected hypodermically and subsequently the dog was etherized. A cannula was introduced into the pancreatic duct and one into the jugular vein of the animal. The mucosa was scraped from approximately 5 feet of the upper part of the intestines of two dogs for each experiment. This was hashed in a hashing machine and secretin prepared according to the method of Bayliss and Starling.[1] To the hashed mucosa were added 200 cc. of 0.4 per cent hydrochloric acid. The mixture was boiled and neutralized while boiling with 1 per cent sodium hydroxide. On filtering a clear filtrate was obtained. This was injected into the jugular vein of the etherized dog 5 cc. at a time at intervals of about five minutes. In this manner 100 cc. of perfectly clear pancreatic juice were obtained in the course of six hours.

The proteolytic activity of this juice was tested by adding a Mett's tube 1 mm. in diameter, 2 cm. in length, containing 10 per cent gelatine colored with Congo red. After standing at room temperature for forty-eight hours there was no indication of digestion.

[1] Starling: Principles of Human Physiology, 1912, 717.

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A typical experiment may be described as follows: 100 cc. of pancreatic juice were collected according to the method given and divided into two portions of 50 cc. each. To one portion were added several crystals of thymol and 2 cc. of enterokinase. The enterokinase was prepared in the following way. A piece of intestine was slit open and thoroughly washed with tap water. The mucosa was gently scraped with the handle of a scalpel. This scraping was extracted with 25 cc. of 0.7 per cent sodium chloride. This solution was centrifugalized and filtered. In this way a fairly clear solution was obtained which possessed no proteolytic activity but was able to activate the trypsinogen solution.

The 50 cc. of juice to which the 2 cc. of enterokinase had been added was placed in the ice chest together with the 50 cc. to which no activator had been added. After standing in the ice chest for twenty-four hours the two solutions were tested for proteolytic activity by adding a Mett's tube of the type described above to 5 cc. of each of the solutions. It was found that the activated juice digested 5.2 mm. of gelatine in forty-eight hours at room temperature. The non-activated juice digested none of the gelatine.

The object of the experiments to be described was to determine if the solution of trypsinogen were more easily oxidized than the solution of trypsin. The 50 cc. of pancreatic juice to which 2 cc. of enterokinase had been added was the trypsin solution and the 50 cc. to which no enterokinase had been added was the trypsinogen solution. Oxidation of the trypsinogen and trypsin was brought about by the oxygen liberated by the passage of the direct electric current through their solutions.

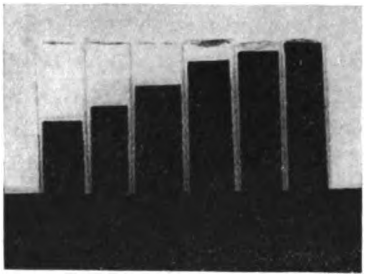
TRYPSIN

Five cubic centimeters of the activated pancreatic juice were introduced into an electrolytic cylinder,[2] a description of which had already been published in this JOURNAL. This cylinder was placed across the electrodes of a direct electric circuit in

[2] Burge: This Journal, 1913, xxxi, 328.

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series with a potential reducer and a milliammeter. The cell was then fastened in a shaking machine and shaken at the rate of 250 double shakes per minute in order to prevent polarization while 25 milliamperes were passed for twenty-five minutes. The electrolyzed trypsin solution was then removed and 5 cc. of fresh trypsin solution introduced. The cylinder was replaced in the shaking machine and 25 milliamperes were passed for fifty minutes. At the end of this period this solution was removed and the cylinder recharged. Electrolyses were continued in this manner until trypsin solutions were obtained through which 37.5, 75, 112.5, 150 and 187.5 coulombs of electricity had passed. When the series was complete a Mett's tube was introduced into each of the electrolyzed trypsin solutions and into 5 cc. of the non-electrolyzed as a control. These solutions stood at room temperature for forty-eight hours when the Mett's tubes were removed and photographed. The reproduction of a photograph of the tubes of a typical experiment represents them approximately twice the actual size (fig. 1). The dark portion represents the amount of undigested gelatine; the light shows the empty tube from which the gelatine had been digested.



I II III IV V VI

Fig. 1. Photograph of Mett's tubes. The dark portion represents the undigested gelatine; the light, the extent of digestion.

TABLE I

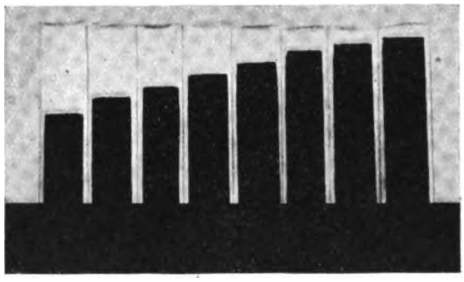
	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	5.0		
Electrolyzed solution.....	II	37.5	4.0	1.0	20.0
Electrolyzed solution.....	III	75.0	2.8	2.2	44.0
Electrolyzed solution.....	IV	112.5	1.8	3.2	64.0
Electrolyzed solution.....	V	150.0	0.5	4.5	90.0
Electrolyzed solution.....	VI	187.5	0.0	5.0	100.0

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The extent of digestion in the same Mett's tubes, expressed in millimeters, is shown in the accompanying table (Table I). It may be seen that the amount of gelatine digested was decreased in proportion to the number of coulombs passed and that the passage of 187.5 Q. completely destroyed the activity of the trypsin.

TRYPSINOGEN

Trypsinogen solutions were electrolyzed in the manner described for trypsin. A series was obtained through which 37.5, 75, 112.5, 150 and 187.5 coulombs had passed. Three drops of enterokinase were added to each of the electrolyzed trypsinogen solutions and to 5 cc. of the non-electrolyzed for a control. These solutions were placed in the ice chest for six hours in order that the trypsinogen might be converted into trypsin. At the end of this time they were removed from the ice chest to room temperature and a Mett's tube introduced into each. After standing at room temperature for forty-eight hours the Mett's tubes were removed and photographed. The accompanying photograph shows the tubes of a typical experiment (fig. 2). Table II gives data regarding this same set of tubes. The extent of digestion in the different tubes is expressed in millimeters and the percentage decrease in digestive strength is given for the number of coulombs passed through the respective solutions.



I II III IV V VI VII VIII

Fig. 2. Photograph of Mett's tubes magnified twice. The dark portion represents the undigested gelatine; the light, the extent of digestion.

The conclusion to be drawn from these experiments is that trypsin is more easily destroyed by the passage of the direct electric current than trypsinogen and since the destruction of both of these substances is due to oxidation the further con-

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clusion is justified that trypsin is more easily oxidized than trypsinogen.

TABLE II

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	5.8		
Electrolyzed solution.....	II	37.5	4.5	1.3	22.4
Electrolyzed solution.....	III	75.0	3.8	2.0	34.4
Electrolyzed solution.....	IV	112.5	3.0	2.8	48.2
Electrolyzed solution.....	V	150.0	2.3	3.5	60.3
Electrolyzed solution.....	VI	187.5	1.5	4.3	74.1
Electrolyzed solution.....	VII	225.0	1.0	4.8	82.7
Electrolyzed solution.....	VIII	262.5	0.6	5.2	89.6

PEPSIN AND PEPSINOGEN

Experiments similar to those carried out using trypsin and trypsinogen were performed using pepsin and pepsinogen.

A typical experiment may be described as follows: Immediately after death the stomachs of two dogs were removed, slit open and washed thoroughly with tap water. These were immersed in a 1 per cent sodium bicarbonate solution for one minute in order to remove any pepsin still adhering and at the same time to harm the pepsinogen as little as possible. Langley and Edkins[3] have shown that pepsin is more easily destroyed by sodium bicarbonate than is pepsinogen. The stomachs were again washed with tap water and rinsed with distilled water. The mucosa was torn off, hashed in a hashing machine and ground up with sand in a mortar. Approximately 100 cc. of 0.7 per cent solution of sodium chloride were added to the hashed mucosa. This mixture was placed in the ice chest for twenty-four hours. At the end of this time it was placed in a press and the liquid portion removed. This liquid was centrifugalized and subsequently filtered through several thicknesses of fine grained filter paper by means of a vacuum pump. The solution thus obtained was moderately clear. This was

[3] Langley and Edkins: Journal of Physiology, 1886, vii, 371.

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divided into two portions of 50 cc. each. To one portion was added 0.5 cc. of a 37 per cent solution of hydrochloric acid while the material was being shaken vigorously. This acid solution and the remaining 50 cc. were placed in the ice chest. After six hours both the activated and the non-activated extract of the gastric mucosa were placed in collodion diffusion tubes and dialyzed against running tap water for twenty-four hours. Each diffusion shell was then suspended in a vessel containing 10 liters of distilled water and dialyzed for twenty-four hours. At the end of this period both tubes were placed in one vessel containing 10 liters of distilled water and dialyzed for another period of twenty-four hours. The result of this treatment was that the conductivity of the two solutions was practically the same.

PEPSIN

The extract of the gastric mucosa to which hydrochloric acid had been added and which was subsequently dialyzed was the pepsin solution used. This solution was electrolyzed in the same manner as the trypsin and trypsinogen solution. Thus

TABLE III

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTED	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	10.0		
Electrolyzed solution.....	II	36	4.0	6.0	60.0
Electrolyzed solution.....	III	72	1.0	9.0	90.0
Electrolyzed solution.....	IV	108	0.0	10.0	100.0

pepsin solutions were obtained through which 36, 72, and 108 coulombs of electricity had been passed. It may be recalled that these solutions had been made practically free of hydrochloric acid by means of dialysis, hence it was necessary to make the electrolyzed pepsin solutions acid before digestion could be begun. For this purpose 1 cc. of 1 per cent hydrochloric acid was added to 3 cc. of each of the electrolyzed pepsin solutions and to 3 cc. of the non-electrolyzed as a control. A Mett's tube was

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introduced into each of the 0.25 per cent hydrochloric acid-pepsin solutions. These solutions were permitted to stand at room temperature for forty-eight hours. The number of millimeters of gelatine digested in that period in the Mett's tubes may be seen in the accompanying table (Table III).

It may be seen from the table that 10 mm. of gelatine were digested by the non-electrolyzed pepsin solution in forty-eight hours, and that no digestion had taken place at this time in the tube in the solution through which 108 coulombs of electricity had passed.

PEPSINOGEN

The extract of the gastric mucosa freed from hydrochloric acid and pepsin by means of 1 per cent sodium bicarbonate and subsequently dialyzed was the pepsinogen solution used. Electrolyses were carried out until solutions of pepsinogen were

TABLE IV

	TUBE	COULOMBS PASSED	MM. GEL. DIGESTED	DECREASE IN MM. GEL. DIGESTION	PERCENTAGE DECREASE IN DIGESTION
					per cent
Non-electrolyzed solution.....	I	0	7.0		
Electrolyzed solution.....	II	36	6.5	0.5	7.1
Electrolyzed solution.....	III	72	6.0	1.0	14.2
Electrolyzed solution.....	IV	108	5.0	2.0	28.5

obtained through which 36, 72 and 108 coulombs of electricity had passed. These pepsinogen solutions were then activated by the addition of 1 per cent hydrochloric acid. One cc. of the acid was added to 3 cc. of each of the electrolyzed pepsinogen solutions and to 3 cc. of the non-electrolyzed pepsinogen solution to serve as a control. These solutions were placed in the ice chest for six hours. During this time the pepsinogen was converted into pepsin. A Mett's tube was then introduced into each of the activated solutions and digestion allowed to proceed at room temperature. After forty-eight hours the tubes were removed and the extent of digestion measured. Table IV gives the measurements for a typical experiment.

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It may be seen that 7.0 mm. of gelatine were digested in the non-electrolyzed control solution in forty-eight hours and that in the solution through which 108 coulombs had passed digestion had taken place to the extent of 5.0 mm.

It will be seen from these experiments that pepsin is more easily destroyed by the passage of the direct electric current than is pepsinogen and since the destruction of both of these substances by the passage of the current is due to oxidation we may conclude that pepsin is more easily oxidized than pepsinogen.

DISCUSSION

Lillie[4] and others have shown that the mucosa of the stomach and of the intestine possesses intense oxidative properties. We[5] have been able to show that all the ordinary digestive enzymes are easily oxidized by nascent oxygen. On the basis of these two facts we have advanced the hypothesis that the mucosa of the digestive tract maintains its integrity during life by rendering inactive the layer of enzyme solution immediately in contact with it. This assumes that there are two opposing activities at work, viz., the active enzyme within the lumen of the digestive tract attempting as it were to digest the cells of the mucosa while the oxidative processes of these cells are rendering the enzyme inactive and hence protecting the mucosa from digestion.

It has been shown that in diseases of the circulatory and respiratory systems,[6] in acute yellow atrophy of the liver and in chloroform and phosphorus poisoning the tendency of the tissues to undergo auto-digestion is greatly increased. It has also been shown that under these abnormal conditions the oxidative processes of the tissues are decreased.[7] The observation has frequently been made that if the blood supply be cut off from a circumscribed area of the stomach wall the pepsin of the gastric

[4] Lillie: This Journal, 1902, vii, 413.

[5] Burge: Ibid., 1914, 141, 146.

[6] Schlesinger: Hofmeiter's Beitrage, 1904, iv, 87.

[7] Welsch: Archives internationales de Pharmacodinamie et de Therapie, 1905, xiv, 211.

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juice begins to digest this area. It is obvious that in such cases, the blood supply being cut off, the area is deprived of oxygen and hence pepsin is left free to act. Under such circumstances gastric ulcers may arise.

If one accepts the hypothesis that a balance normally exists between the digestive enzymes in the lumen of the alimentary tract and the oxidative processes of the cells lining this tract it is necessary to explain the fact that pepsinogen is not oxidized during the process of its secretion by the cells of the gastric mucosa and the fact that trypsinogen is not oxidized by the cells of the pancreas since the cells of both of these tissues possess oxidative properties. The explanation that suggests itself is that pepsinogen and trypsinogen are not so easily oxidized as the active pepsin and trypsin. The foregoing experiments prove that this is true.

CONCLUSIONS

1. Trypsin is more easily oxidized than trypsinogen.
2. Pepsin is more easily oxidized than pepsinogen.
3. The fact that trypsin and pepsin are relatively easy to oxidize makes it possible for the mucosa of the stomach and intestine to protect itself from digestion by means of its oxidative processes.
4. The fact that trypsinogen and pepsinogen are relatively difficult to oxidize prevents these substances from being oxidized in the cells of the pancreas and of the gastric mucosa during the process of secretion.

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THE EFFECTS OF EPINEPHRIN INFUSION ON VASOMOTOR IRRITABILITY

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That adrenal deficiency results in vasomotor depression is well known. Two explanations of the phenomenon have been offered which assume that the essential factor is a reduction in quantity of circulating epinephrin. One—the "tonus" theory—supposes that the sympathetic system under normal conditions is kept in continuous activity by epinephrin stimulation. This theory is no longer tenable(1). Another possible explanation was proposed by Elliott in 1904 (2)—namely, that in the absence of epinephrin the ability of the "myoneural receptive substance" to transmit impulses from the sympathetic fibres to the muscle cells is lessened. In a previous research on partial adrenal deficiency (3) occasion was offered to test the effect of adrenalin infusion in two dogs showing vasomotor depression. The surprising fact was noted that while the animals were under the influence of small quantities of epinephrin the responses to vasomotor stimulation were actually depressed. The phenomenon seemed worthy of further investigation.

Accordingly we have studied the effects of adrenalin infusion upon vasomotor irritability in 42 other dogs. The animals were all anesthetized,—various methods being used: ether alone by open cone or by the ether-bottle, ether-urethane, and paraldehyde by stomach, supplemented when necessary by ether. The most satisfactory method, however, was decerebration. In some cases curare was injected by vein, particularly when sciatic stimulation was used, in which case it added materially to definiteness in results. In such instances artificial respiration of course had to be employed. For this purpose the Gesell

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and Erlanger apparatus was used. Several of the earlier experiments were made upon dogs partially or completely deprived of their adrenal tissue. This procedure made no apparent difference in results, however, and in all the later cases was omitted. This finding incidentally is in harmony with our previous experience which indicates that aside from shock adrenal extirpation produces no immediate effects whatever. It supports our present belief that epinephrin in significant amount ordinarily is not present in the blood stream. The condition of the vasomotor apparatus before, during and after the adrenal infusion was tested by various methods of stimulation: faradization of the sciatic nerve and of the left splanchnic nerve; and intravenous injections of nicotin, adrenalin and pituitrin. The adrenalin for infusion was diluted with distilled water usually to a concentration of 1:100.000, but various other dilutions from 1:10,000,000 to 1:25,000 were also tried. The infusions were made from a burette connected with a hypodermic needle inserted in a vein—usually an external jugular. The rate of flow was regulated by raising and lowering the burette. Blood pressure was recorded from a femoral artery by the reservoir cannula method previously described(4).

While the results of the series of experiments as a whole are in harmony with our earlier findings, a great deal of variability was noted. In some instances the merest trace of epinephrin added to the blood stream was sufficient to cause a marked depression of vasomotor irritability; in other instances comparatively large quantities were required to cause any material decrease in reactions. In these later cases the augmented pressure due to the adrenalin might in itself, if they stood alone, be regarded as the sole cause of decreased reaction to further stimulation. A considerable number of animals were intermediate between the two extremes. For instance, a given dose of nicotin might cause a rise of 40 mm. of pressure. If now adrenalin was infused at a rate to raise the general pressure level 20 mm., a repetition of the dose of nicotin would cause a further rise of 20 mm., but no summation. When the adrenalin was discontinued the nicotin again produced its original effect, 40 mm.

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rise. In other words the nicotin stimulation plus the stimulation due to the infused adrenalin was no greater than that of the nicotin alone. That there is a depressing factor involved in such results is shown by the fact that when a given dose of adrenalin and of nicotin were mixed and injected together a greater effect was produced than that due to either acting alone. It follows, therefore, that the effect of adrenalin infusion plus nicotin injection is a resultant of three factors: nicotin stimulation, adrenalin stimulation and adrenalin depression of irritability the depression developing more slowly than the stimulation. In a specific case, as a matter of fact, it was found to require

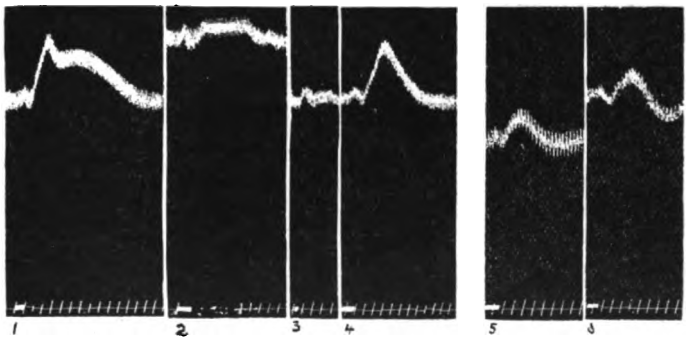


Fig. 1. Adrenalin infusion, nicotin stimulation. At 1, 2, 3, 4, 5 and 6, 2 cc. nicotine 1:4000, injected by femoral vein. 1, normal; 2, during adrenalin infusion; 3, during adrenalin infusion and amyl nitrite inhalation; 4, normal; 5 and 6, later experiment, same animal; 5, during amyl nitrite; 6, immediately after. Infusion adrenalin 1:100,000 by jugular vein, 2.5 cc. per minute. Blood pressure from femoral artery. Dog weight 5 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.) Reduced to ½.

about three minutes for the depression to develop after the infusion began, although usually it occurred almost immediately.

The confusing condition in such cases is of course the rise of pressure that accompanies the epinephrin infusion. It is possible, however, by the use of amyl nitrite to counteract this pressor effect, leaving the depressor influence plainly in evidence. Figure 1 illustrates such a case. The animal was given 2 cc. of nicotin, 1:4000, by vein. The result is shown in graph 1. Then

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adrenalin, 1: 100,000, was infused at the rate of 2.5 cc. per minute. After one and one-half minutes infusion a repetition of the nicotin produced almost no effect (graph 2). While the adrenalin was still flowing the animal was made to breathe amyl nitrite to bring the pressure down to the original level and the nicotin injection repeated. Again the nicotin produced little effect (graph 3). The interval between injections was three minutes. The amyl nitrite was discontinued and the pressure again rose to the height shown in graph 2. The adrenalin infusion was then discontinued and the pressure fell to the original level. One and three-fourths minutes after the adrenalin was stopped a repetition of the nicotin injection gave its original effect (graph 4). That the amyl nitrite was not itself the cause of the block in graph 3 is shown by graphs 5 and 6 obtained later from the same dog. They show the effects of the same dose of nicotin during and after amyl nitrite inhalation. There is no appreciable difference in the two cases.

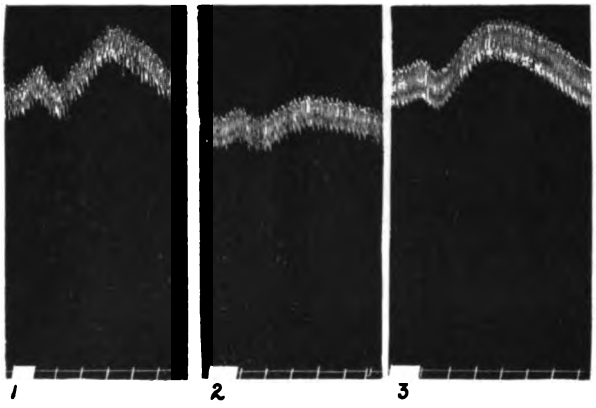


Fig. 2. Adrenalin infusion, nicotin stimulation. Nicotin, 1:4000, 1 cc. by femoral vein. 1, before; 2, during, and 3, after adrenalin infusion, 1: 100,000, 2.6 cc. per minute by jugular vein. Blood pressure from femoral artery. Dog weight 9 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.). Reduced to ½.

Figure 2 illustrates the convincing results obtained in an animal that was unusually sensitive to adrenalin. Graph 1 shows the effect of 2 cc. of nicotin, 1:4000. Adrenalin infusion 1:200,000 at the rate of 2.6 cc. per minute caused a slight lowering of the general blood pressure level. Two and one-half minutes after the infusion began the nicotin stimulation was repeated and

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caused only a slight rise (graph 2). When the adrenalin was discontinued the pressure returned to its previous level and again (graph 3) nicotin caused the same rise as before.

That the depression of irritability is a specific effect of the adrenalin was shown in another way. In an animal that gave a clean-cut decrease in the nicotin reaction during adrenalin infusion the conditions were reversed. Nicotin was used as the infusion agent, a rate being selected that closely simulated the effect of the adrenalin infusion. Adrenalin was injected as the stimulating agent, before, during and after the infusion. No depression of the reaction occurred.

Having established the fact that epinephrin produces a depression of vasomotor irritability an attempt was made to determine the locus of the block. Different components of the vasomotor apparatus were stimulated by appropriate means and the effects of adrenalin infusion noted.

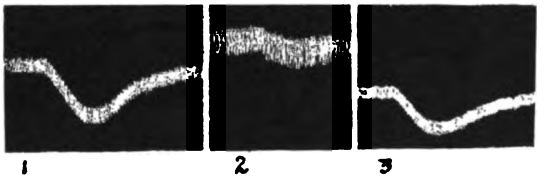


Fig. 3. Adrenalin infusion, sciatic nerve faradized. Stimulation at 1, before; 2, during, and 3, after infusion with adrenalin, 1:50000 2.3 cc. per minute, by jugular vein. Interval between stimulations, 2 minutes. Blood pressure from femoral artery. Dog weight, 7 kilos. Anesthesia, ether. Initial blood pressure 87 mm. No reduction.

As an example of the afferent nervous components the sciatic was selected. This was exposed through an incision in the posterior aspect of the leg at the mid-femoral region. A Harvard shielded electrode was applied and packed off with dry cotton. A tight ligature was tied around the nerve distal to the electrode and the incision closed. In some cases curare was administered and in others not; the results were qualitatively the same in both cases but quantitatively greater in the curarized dogs. The nerve was stimulated by induced currents of various degrees of strength to produce in some instances a pressor and in others a depressor effect. The proper position of the secondary coil having been determined in each instance the effect of stimula-

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tion before, during and after adrenalin infusion was determined. When pressor stimulations were employed results closely similar to those obtained with nicotin were secured. These therefore need not be further described. Figure 3 show the results of an experiment in which depression resulted from the stimulation. The rate of adrenalin infusion (1:50,000) was 2.3 cc. per minute. This caused a slight rise of the pressure level. The intervals between stimulations were two minutes. The graphs show the effects of stimulation (1) before, (2) during and (3) after infusion. The sciatic nerve experiments as a whole showed that both the pressor and depressor mechanisms are blocked and indicate that the results obtained in the nicotin injections are

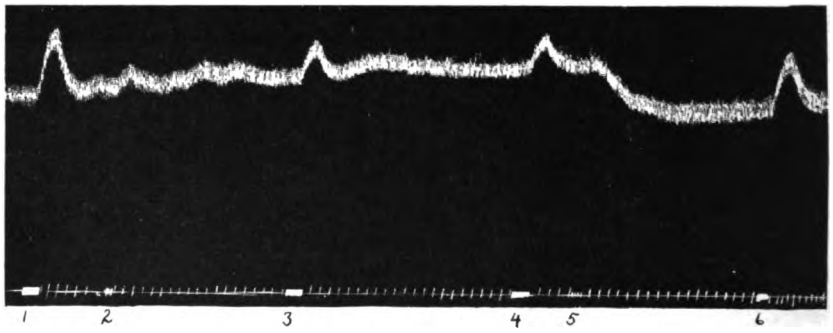


Fig. 4. Adrenalin infusion, splanchnic nerves faradized. At 1, 3, 5 and 6 stimulation. At 2, infusion begun. At 5, infusion discontinued. Adrenalin 1:100,000, 5.7 cc. per minute. Blood pressure from femoral artery. Dog weight, 11 kilos. Anesthesia, ether. Base line: 0 pressure, signal and time (5 secs.). Reduced to ½.

not to be ascribed to any sort of chemical interaction among the drugs employed.

Figure 4 shows the results obtained in an experiment in which stimulus was applied to the peripheral (post-ganglionic) nervous elements. In this case the left splanchnic trunks were isolated in the region of the adrenal gland. The adrenal was tied off. A shielded electrode was adjusted to the nerves which were tightly ligated centrally. The electrode was surrounded with dry cotton to absorb any fluid that might accumulate and

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the incision closed. At 1 a faradic current was sent through the electrode. At 2 infusion with adrenalin 1:100,000 was begun. At 3 and 4 the stimulation was repeated. At 5 the adrenalin was discontinued and at 6 the stimulus was repeated. The same strength of current of course was used throughout. The reaction was depressed about 50 per cent. Other experiments gave similar results.

According to Pilcher and Sollman's late work (6) nicotin stimulates the vasomotor centre but according to Langley and Dickinson (7) the effect is chiefly on the ganglia of the sympathetic system. The foregoing evidence as a whole indicates that adrenalin depression is shown when the stimulus is applied to any part of the vasomotor system proper. This fact indicates that the block is in part at least peripheral, either in the myoneural junctions or the smooth muscle itself. The fact, however, that both the pressor and depressor mechanisms are affected is most easily explained on an assumption that the depression is partly central (compare figs. 2 and 3).

Our attempts to settle the point by direct experiment were not entirely successful. In several cases both nicotin and adrenalin injections were made during adrenalin infusion. Usually the nicotin reaction was more depressed than was the adrenalin reaction, but depression was shown in both cases. Such findings are hard to interpret because the adrenalin stimulation was effected through the myoneural junction, a structure that was already undergoing excitation by the infused adrenalin, whereas, in case of the nicotin stimulation the myoneural junction was merely transmitting the impulses. To what extent the transmission involves also excitation is undetermined. In a single instance we have noted in the course of another research not yet published that myoneural transmission may remain unimpaired when myoneural excitability is markedly decreased. To state the case specifically, it was noted that after injection with a thyroid preparation an animal lost the greater part of its irritability to adrenalin while the reactions to pituitrin and to nicotin were unaffected.

The reaction to pituitrin which stimulates the smooth muscle

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only was also investigated. Several difficulties presented themselves in such experiments. Repeated doses of pituitrin have to be small or they soon cease to be effective, and with a small initial stimulus the effects of adrenalin infusion were difficult to demonstrate. Also, the doses can not be repeated at short intervals as can adrenalin and nicotin and the condition of the experimental animal is likely to change from one injection to the next. Comparative studies of the relative effects of the infusion upon the pituitrin and the other reactions are not conclusive because pituitrin itself, according to Keponow (8), alters the excitability of the vasomotor mechanism. So far as could be judged, however, in the face of these difficulties, the vasomotor reaction to pituitrin is also somewhat depressed by adrenalin infusion. The depression therefore is probably both central and peripheral.

Our first experiments were made to determine whether adrenalin has any ability to *facilitate* sympathetic functioning. Considering the fact that various instances of reversal of effect have been reported with change of dosage it was not *a priori* unlikely that very high dilutions might augment irritability even in those animals that showed depression with greater quantities. In the first experiments of this series the matter was investigated. The infusions were begun with very dilute solutions, e.g., 1:20,000,000, and the strength gradually increased by stages to an effective concentration. In one or two instances there was noted slight augmentation in the reactions to stimulation but this was within the limits of experimental error. Ordinarily the first effect to be seen was depression. It appears therefore that epinephrin has little or no ability to increase vasomotor irritability or the transmission of vasomotor impulses.

The vasomotor depression which was noted developed quickly after the epinephrin infusion began, and was as quickly recovered from when the infusion was stopped. In one instance a lag of two or three minutes was noted but generally the lag was not more than half a minute.

That reflex discharge of epinephrin from the animal's own glands is not a significant complicating factor in our results is

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indicated by three facts: the results in several instances in which the animal's glands were removed were smiilar to those in normal animals; the latent period after stimulation was shorter than that necessary for the stimulus to produce its effects *via* the adrenals; and usually, the secondary waves in the pressure curve that are characteristic of adrenal discharge did not occur. Moreover, in most cases a secondary discharge of epinephrin would have made no difference in the significance of the results.

Our results are possibly merely a phase of the depressor influence of epinephrin recently studied by Hoskins and McClure (9) in dogs and by Cannon and Lyman (10) in cats. These investigators have corroborated previous reports that under suitable conditions of dosage and rate of injection adrenalin characteristically produces a fall of blood pressure. Cannon and Lyman are inclined to attribute the depression to an influence on the vascular muscle cells. In a preceding paragraph we called attention to evidence that the depression is partly central, but our results also indicate a peripheral effect.

As to the significance of our findings we have little comment to offer. They are quite at variance with what we had first expected. They are scarcely reconcilable with any of the more popular theories as to the functions of the chromaffin system. They seem to indicate that the presence of small quantities of epinephrin in the circulating blood is not only of no use to the individual but in many cases at least is actually detrimental. If this be true it is quite probable that under ordinary conditions epinephrin does not exist in the blood at all. Under conditions of special stress, however, when the adrenal glands are discharging relatively large amounts of their secretion, the depressing influence as compared with the stimulating effect would be slight and probably neglible.

SUMMARY AND CONCLUSIONS

1. In 44 anesthetized dogs the effects of adrenalin infusion upon the irritability of the vasomotor mechanism was investigated.

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2. The vasomotor mechanism was stimulated by faradization of the sciatic and splanchnic nerves, and by injections of nicotin, adrenalin and pituitrin, before, during and after intravenous infusion with adrenalin.

3. No concentration of adrenalin gave satisfactory evidence of augmenting vasomotor irritability, or facilitating the transmission of vasomotor impulses.

4. In most cases the infusion lessened the vasomotor irritability—sometimes to a marked degree.

5. The animal's own adrenal glands played no significant part in the results.

6. The irritability of both the pressor and depressor mechanisms was decreased.

7. The depression was probably both central and peripheral.

8. Circulating epinephrin is probably not a factor in the ordinary functioning of the animal economy.

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THE DISTRIBUTION OF GASTRIN IN THE BODY

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A. INTRODUCTION AND LITERATURE

Since the almost simultaneous reports in 1906 by Edkins and Gross that a gastric secretin probably is a factor in stimulating gastric juice secretion further work on this phase of secretagogue action had not been reported until recently. This later work was not known to us and most of it had not been published at the time these investigations were begun in the summer of 1912.

Edkins[1] demonstrated that 0.4 per cent hydrochloric acid extracts of the cardiac or pyloric mucous membrane from hogs' stomachs when intravenously administered to an anesthetized cat cause a secretion of gastric juice, but that similar extracts from the fundus portion are inactive. He further stated that extracts prepared by using cold water, peptone, glucose, or glycerol solutions as solvents also contain variable amounts of this same secretagogue which he called gastrin. Gross[2] in the Pawlow laboratory also suggested that a gastric hormone may be involved in gastric secretion in that he found beef extract to cause a flow of gastric juice only when introduced into the pyloric part of the stomach, and not when introduced into the fundic part. Popielski[3] in a series of individual and joint papers repeatedly

[1] J. S. Edkins: Jour. of Physiol, 1906, xxxiv, 133.

[2] Walter Gross: Arch. f. Verdauungskrankheiten, 1906, xii, 507.

[3] L. Popielski: Pflüg. Arch., 1909, cxxvi, 483; ibid., 1909, cxviii, 191; L. Popielski and K. Panek; ibid., 1909, cxxviii, 222; L. Popielski: Centrbl. f. Physiologie, 1910, xxiv, 635; ibid., 1910, xxiv, 1102; Centrbl. f. Biochem. u. Biophys. 1910–11, xi, 724; Pflüg. Arch., 1912, cxliv, 135; Pflüg. Arch., 1913, cl, 1.

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calls attention to the general distribution in tissues and in Witte's peptone of a substance or of substances which cause vasodilation when introduced intravenously; also that coincident with the fall in blood pressure we have a decreased coagulability of the blood and a more active secretion in the digestive glands in general. He considers the secretagogue action as due not to a specific effect on the cells, but to the more fluid (more "filterable") condition of the blood as well as to the vasodilatation. This substance or these substances so generally distributed he called "vasodilatin." He reported finding vasodilatin in brain, gastric and intestinal mucosa, pancreas, defibrinated blood, extracts from erythrocytes and in Witte's peptone. He found the hetero-, deutero- and proto-albumose fractions free from the substance and considers the activity due to a hydrolytic product from the proteoses or peptones. He does not report detailed comparative quantitative studies on the effects of these extracts on gastric secretion. Thus in his comparative physiological study on the effects of these various extracts he reports three experiments showing that 5 to 6 cc. of 5 per cent extracts in 0.4 per cent hydrochloric acid from the mucous membrane of the large intestine and rectum when injected intravenously (subcutaneously in one experiment) into dogs with oesophageal and gastric fistulas, caused an increased flow of fluid from the stomach as high as 18 cc. in one-fourth of an hour, but in no case was the acidity or peptic activity reported as measured before or after the injection. He does not report the effect of a similar extract from the gastric mucosa, but repeatedly states that large doses of peptone, intestinal or gastric mucosa extracts not only do not stimulate the flow of gastric juice, but may actually inhibit it. He criticizes Edkin's negative findings with fundus mucosa extract as possibly due to an overdose of "vasodilatin." In his most recent paper Popielski again calls our attention to the same general action of tissue extracts and also to the fact that increased submaxillary secretion is always accompanied by a decreased coagulability of the blood flowing from the gland, no matter whether the gland is stimulated to secretion by acid in the mouth, by pilocarpine or by stimulation

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of the chorda tympani. In this connection it is interesting to note Popielski's statement that although imid-azol ethylamine (Modrakowski[4]), large doses of atropin, or stimulation of the depressor nerve cause a fall in blood pressure, still we do not observe a decreased coagulability of the blood or an increased secretion by the digestive glands. On the other hand, that when decreased coagulability of the blood accompanies a fall in blood pressure as in the action of vasodilatin, in blood transfusion or in anaphylaxis we do have an increased secretion by these glands. Popielski has not separated vasodilatin in pure form, but considers the substance to be different from cholin. From the researches of Popielski and his coworkers we are not able definitely to state what the factors are which are involved in secretagogue action; we are not able to state definitely that vasodilatin is the secretagogue, nor are we able to say whether the action is due to one substance which is present in different concentrations in various tissues, or whether to different substances with quantitatively different specificities, but not absolutely specific in any case.

A report by Mironescu[5] on intestinal secretion stimulation is of interest in this connection. A qualitative study led him to conclude that acid extracts from the rectum, large and small intestines, oesophagus, fundus, pylorus, duodenum, salivary gland, liver and suprarenals cause intestinal secretion in a Thiry-Vella fistula dog. Similar experiments from brain, pancreas, muscle or heart and peptone solutions he found inactive. The actual rate of flow and the character of the juice obtained were not considered in these studies, a more rapid flow for a short period of time was taken as a positive response. It is hardly necessary to state that studies on stimulation of intestinal juice secretion involve many complications and that a positive finding may easily be due to indirect secondary stimulations. Eisenhardt[6] has in part confirmed Edkins' findings. He employed

[4] G. Modrakowski: Arch. f. exper. Path u. Pharm., 1912, lxix, 67.

[5] Mironescu: Inter. Beitr. z. Path. u. Therap. Ernährungstörungen, 1910, i, 194.

[6] W. Eisenhardt: Inter. Beitr. z. Path. u. Therap. d. Ernährungsstörungen, 1910, i, 358; ibid, 1910, ii, 203.

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dogs with Heidenhain-Bickel accessory stomachs and found the injection of dog gastric juice to cause a distinct but short rise in gastric secretion, providing the juice injected had been collected from a gastric fistula. Juice collected from the accessory fundus stomach possessed no secretagogue action. So also juice collected from the entire stomach when digested with casein or lactalbumin for one to five hours no longer possessed the hormone action, but if digested longer than five hours it was again found active. The fundus juice, however, could not be made active by such treatment, nor by more prolonged incubation with the protein. He reports negative results with the subcutaneous injection of predigested proteins (by acids), sodium oleate, lactose, glucose, saccharose, sodium chloride, commercial pepsin and gliadin digested thereby. On the other hand whey from milk and the dialysate from the whey reacted positively; a water extract from horse flesh reacted doubtfully, but a similar extract from roasted barley or wheat was followed by a good response; a press juice from spinach caused a very marked secretion. The author does not give quantitative data as to actual yield or nature of juice, nor as to the concentrations or amounts injected. R. Ehrmann[7] finds subcutaneously injected extracts from pyloric, fundic and duodenal mucosa in $\frac{N}{10}$ hydrochloric acid to cause a flow of gastric juice in Pawlow stomach dogs. He could not definitely distinguish differences as to degrees of activities due to marked variations and irregularities in the results. The maximum effect was noted in fifteen to thirty minutes after the injection. He reports negative results with Witte peptone, sodium nucleate, sarcosin, creatinine, alanin and glycocoll, Liebig's meat extract in doses of 0.5 to 1.0 gram were without effect, but a dose of 6.2 gram caused gastric secretion.

Otto Emsmann[8] from studies on dogs with Heidenhain pouches considered the gastric secretin to be present in the acid extracts obtained from the pylorus, duodenum, jejunum-ileum, liver and

[7] R. Ehrmann: Inter. Beitr. z. Path. u. Therap. d Ernährungsstörungen, 1911–1912, iii, 382.

[8] Otto Emsmann: Inter. Beitr. z. Path. u. Therap. d. Ernährungsstörungen, 1912, iii, 117.

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pancreas, but mainly in the pylorus, duodenum and liver, the liver extract being very nearly as active as the pylorus extract. Emsmann's methods as reported are not quantitative enough to definitely establish this. The total secretion after an injection and the character of the secretion were not determined.

Z. Tomaszewski[9] in a preliminary report finds extracts from pyloric and fundus mucosa to cause a flow of gastric juice when administered to vagotomized dogs with gastric and duodenal fistulas. He studied quantitatively the quantity and acidity of the juice obtained; the peptic activity was not reported. B. E. Maydell[10] found extracts from the pyloric mucous membrane when subcutaneously administered to chronic gastric fistula dogs, to cause a flow of gastric juice; the amounts varied from 12½ to 387 cc. per hour, the period of collection being one to one and one-half hours after injection. The acidity of the juice thus obtained was in general of the same order as pyschic juice from oesophageal fistula dogs, but the peptic activity (by Mett method) of the latter juice always was over twice that of the gastrin juice. Maydell reports negative findings with physiological salt solution, neutralized gastric juice, fundus extract and pancreatic secretin. It is hardly necessary to state that the purest preparations of pancreatic secretin thus far reported on have not been found to stimulate gastric secretion and that the pancreatic secretion brought about is of an entirely different character from that brought about by cholin or the so-called vasodilatin.[11, 12] To be sure a secretin solution showing *no* blood pressure lowering action has as yet not been obtained.

B. EXPERIMENTAL METHODS

(*a*) *Preparations.* In the studies cited above no attempts were made to carry out comparative physiological studies with other than the very crudest extracts. It was our purpose to

[9] Z. Tomaszewski: Zentbl. f. Physiol., 1913, xxvii.
[10] B. E. Maydell: Pflüg. Arch., 1913, cl, 390.
[11] Bayliss and Starling: Jour. of Physiol., 1902, xxviii, 325.
[12] Dixon and Hamill: Jour. of Physiol., 1909, xxxviii, 314.

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first find a method which will yield a fairly active and stable preparation and still remove foreign substances, including vasodilatins as much as possible. Having established this we then sought to determine fairly quantitatively the specificity or distribution of the secretagogue in widely different tissues, and also to quantitatively follow the nature and quantity of the secretion obtained.

The method adopted after a number of preliminary experiments is based mainly on observations which have been made on the preparation of extractives in general and particularly on the preparation of pancreatic secretin claimed to be fairly free from vasodilatin. Briefly, the well washed fresh material was hashed and mixed with five times its weight of 0.4 per cent hydrochloric acid, then heated to 90° C. on the steam bath, set aside to cool to room temperature, the next day again heated to 90° C. and after again cooling and standing for twenty-four hours, filtered. The noted volume of recovered filtrate was then concentrated under diminished pressure to $\frac{1}{18}$–$\frac{1}{10}$ its original volume. Next we added six volumes redistilled 95 per cent alcohol, allowed to stand one to three days, filtered and the filtrate evaporated to dryness under diminished pressure. After dehydrating the residue by evaporating three times with 25 to 75 cc. portions of absolute alcohol, the material was extracted three or four times with boiling absolute alcohol. The residue insoluble in absolute alcohol was dissolved in water and again evaporated to dryness under diminished pressure. This treatment with water was repeated two or three times to remove the alcohol. Finally the residue was dissolved in water, filtered and diluted so that 1 cc. represented approximately 4 to 5 grams fresh tissue. This solution was then sterilized three or four times in sealed tubes on successive days. Hog tissues were used throughout.

(*b*) *Animals, methods of injection, dosage.* The advantages of testing the activity of gastrin preparations without the complicating factors of an anaesthetic are so obvious that they need not be dwelt upon. For these reasons it was decided to use dogs having Pawlow accessory stomachs and gastric fistulas.

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This makes it possible to run many preparations on the same test stomach, thus eliminating the variations of the different individuals. The gastric fistula was prepared by establishing an opening through the left rectus muscle into the anterior wall of the fundus in the line of the anastomoses of the arterioles from the two curvatures of the stomach. Such animals maintain themselves in perfect health after the development of a functional sphincter of scar tissue about the fistulous opening. The Pawlow accessory stomachs were prepared and cared for in a manner which has been previously described by one of the authors.[13]

It was important, of course, to have the stomachs empty and secretorily quiescent before an experiment was to be run. Boldyreff[14] has shown that during certain fasting intervals there is a marked secretion of juice. While we have made no attempt to study this phase of the subject, our experience has shown that a fasting stomach frequently displays appreciable activity. Thinking that such activity might be associated with residual food particles in the mucosal folds, we first washed out the stomachs, one or two hours before the experimental periods. This procedure, instead of stopping a low grade secretion, apparently threw the mechanism into a more active state, so that secretion might last one, two, or three hours longer. The plan, which gave the most satisfactory results and which was finally adopted as a routine measure, consisted of feeding the animals heavily thirty-six hours before the experiment, and eighteen hours later giving each a pint of milk.

Animal holders were found to be a necessity, for only in this way can one be certain of the quantities of the juice. The dogs can be made quite comfortable, since they sleep during a good portion of the experimental period. It is important that juice be collected over a control period of two hours before the injection is made, so as to determine whether the trend of the stomach is to a higher or lower secretory level.

[13] R. W. Keeton: This Journal, 1914, xxxiii, 25.
[14] W. Boldyreff: Ergebnisse der Physiologie, 1911, xi, 121.

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Intravenous injections into the leg vein caused some respiratory distress, and proved to be rather transient in their action. The intramuscular administrations into the lumbar region on the other hand presented no such difficulties, so this method was adopted. It is needless to say that with any long series of experiments a rigid aseptic technique and a sharp needle must be employed, if the dogs' backs and dispositions are to be kept in a satisfactory shape. The dosage usually consisted of 1 cc. of the preparations, which was doubled in the cases of very large animals.

C. ANALYTICAL METHODS

The collections of the juice were made hourly. The acidities were determined by titration with $\frac{N}{10}$ NaOH, and the percentages were expressed in terms of HCl as "free acidity" and "total acidity." In estimating the former, dimethyl-amido-azo-benzene was used as an indicator, and phenolphthalein for the latter. The expression of the results in terms of HCl facilitated the calculations for the pepsin estimations.

In estimating pepsin the Mett's tubes were used, under the modifications advised by Cobb.[15] A total volume of 3 cc. consisting of 1.5 cc. of juice and an equal amount of a diluent, was employed. The diluent consisted of the necessary volumes of $\frac{N}{5}$ HCl and water to bring the final acidity of the mixture up to 0.3 per cent HCl. The free acidity of the juice was used as a basis for this dilution. The tubes were incubated for twenty hours, and the peptic activity obtained by adding the millimeters of albumin digested at either end. Following Schütz' law, the square of the digestion in millimeters was multiplied by the number of cubic centimeters of juice secreted in order to obtain the peptic units of a given sample. The peptic activity represents the square of millimeters of albumin digested.

[15] P. W. Cobb: This Journal, 1905, xiii, 448.

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D. RESULTS

1. The nature of the secretory response

An examination of the appended protocol shows the essential characters of the response to gastrin stimulation.

Dog IV. Gastric fistula. Weight 25 kilos

10.30	dressed			
11	11.0 cc. mucus; free acidity 0.000,		Total 0.027	Peptic act. 0.0
11.01	Injected 2 cc. fundus gastrin			
11.15	11.0 cc.	Free acidity 0.018	Total 0.109	32.49
11.30	23.0 cc.	0.319	0.391	8.41
11.45	15.0 cc.	0.419	0.474	5.76
12	13.0 cc.	0.346	0.401	4.84
12.15	9.0 cc.	0.228	0.309	8.41
12.45	1.4 cc.	0.255	0.291	

It will be noted that the latent period of the secretion is ten to fifteen minutes. The maximum quantity is secreted in the second fifteen minute period, and the maximum acidity is reached in the third quarter. When collections were made hourly, the juice of the first collection was usually larger in quantity, but of slightly lower acidity, than that of the second hour. Very rarely do the stimulating effects last longer than two hours, usually one and a half. The rise in acidity follows closely the increase in quantity. Indeed these two factors can be safely relied upon as criteria of the state of the mechanism, a fact which falls in line with Pawlow's contention[16] that the acidity of a given sample of juice is a function of the rate of secretion. This principle has recently been shown to hold for the human stomach by Reyfuss and Hawk.[17]

The pepsin content of a given sample of juice is apt to be rather misleading at first glance. The pepsin concentration may either rise or fall, following the injection of gastrin, depending on the relative quiescence of the glands before the injection, and the amount of fluid secreted. As a rule the fasting secretion of Pawlow stomachs consists of a thick viscid mucous material

[16] J. P. Pawlow: The work of the digestive glands. London, 1902, p. 31.

[17] M. E. Reyfuss, Olaf Bergheim and P. B. Hawk: Jour. Am. Med. Assn., lxiii, no. 24, 2088.

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containing little or no acid, but high in pepsin. If now this type of stomach is stimulated with gastrin, the pepsin concentration falls, while the total number of peptic units secreted rises, which after all is the important thing. The pepsin secreting mechanism coördinates itself less closely with the acid and quantity factors, than do these latter two with each other. At times the former cells appear never to become quite quiescent, and at other times they spring into activity from a stimulus that does not affect the quantity output. This is shown in the preceding protocol. In the first fifteen minutes period the peptic activity was 32.49, and in the second 8.41. In other words, the pepsin production was going on full blast, before the acid and quantity production. Two experiments on a cat with a Pawlow stomach showed the same facts. In the first case, before the injection there was a digestion of 3 mm. (peptic activity 9.0), and after the injection, with no change in quantity of juice secreted, there was a digestion of 8.4 mm. (peptic activity 70.56). In a gastric fistula stomach one is more liable to find the fasting secretion free from residual pepsin, hence a stimulation will show an increase in both the total peptic units and the peptic activity (concentration per cc.). The variability of the factors controlling the pepsin secretion, and the lack of refinement of methods for measuring it, do not warrant an extended discussion of its secretion at present. However, the subject can be rested with this statement, that in all cases an injection of gastrin will increase the total output of pepsin.

2. Comparison of gastric fistula and Pawlow stomachs

At the outset of the work, it soon became evident that the method of response to stimulation of Pawlow and gastric fistula stomachs was different in many respects, and it became necessary to study these points of differences before one felt sure of the interpretation of the results. It is not the purpose of this paper to discuss fully these differences, but a brief summary is necessary for the selection of a physiological preparation which will assay accurately the gastrin samples.

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Gastric Fistula Stomach

In this form of stomach we have the most sensitive possible mechanism. The operative procedure is merely to anchor to the abdominal wall, the stomach at a spot where only the terminations of the nerves and blood vessels are to be found. Indeed this very sensitiveness is rather annoying when experiments are first begun upon an animal. Restlessness and fretting apparently set up a spontaneous secretion which may be of appreciable proportions. This factor necessitated several trial runs in the holders, and injections with tap water before the animal adjusted himself to the routine of the experiment.

There was always noted a distinct summation following two successive injections. This may be a true summation of submaximal stimuli, or it may be evidence that there is an inhibitory mechanism to be dealt with as well as a positive secretive one. The following protocol is typical in its illustration of this point.

TABLE I

Dog VIII. Gastric fistula

TIME	QUANTITY	ACIDITY		PEPTIC ACTIVITY	PEPTIC UNITS
		Free	Total		
10	Dressed				
11	3.0 cc.	0.164	0.291		
12	4.5 cc.	0.018	0.237	28.62	85.87
1	6.5 cc.	0.036	0.191	54.76	237.20
1	Injected 1 cc. pyloric gastrin				
2	11.0 cc.	0.164	0.291	72.25	529.7
3	9.5 cc.	0.300	0.446	68.89	436.3
3	Injected 1 cc. pyloric gastrin				
4	36.0 cc.	0.419	0.492	26.01	624.3
5	23.0 cc.	0.410	0.464	28.09	430.7
6	7.5 cc.	0.300	0.382	32.49	162.3

The quantities secured are much larger than in Pawlow dogs, since the entire stomach output is obtained.

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The contamination of the juice with saliva must be considered, but it is usually negligible. However, the regurgitation of bile and intestinal content at times does become an important element. The volume is probably not altered to any great extent, but the inhibitory influence of the bile makes accurate pepsin estimations impossible.

Pawlow Accessory Stomach

The operative procedure has inevitably damaged the nerves to a considerable extent. Hence the mechanism should not be so sensitive, and this is borne out by actual experience. Fretting and restlessness rarely complicate the secretory picture. The appetite secretion may perhaps manifest itself once or twice when the animal is first handled, but it is more often absent than present. The stomach can be characterized as "secretorily stable."

Usually summation is either absent or not marked. Hence one injection serves very well to get a measurable comparative response from the stomach. This is shown below.

Dog XI. Pawlow accessory stomach

9.35	Dressed			
10.35	0.96 cc.	Free acid 0.072	Total acid 0.164	
10.35	Injected 1 cc. pyloric gastrin			
11.35	6.6 cc.	Free acid 0.373	Total acid 0.418	Peptic act. 21.16
1.10	1.9 cc.	0.155	0.291	31.36
1.16	Injected 1 cc. pyloric gastrin			
2.16	4.8 cc.	Free acid 0.346	Total acid 0.401	32.49
3.30	1.4 cc.	0.273	0.346	

The quantities are small, but there are no complicating factors of saliva, bile, intestinal contents, and possible faulty drainage, which introduce a mental reservation into one's conclusions.

For these reasons the Pawlow stomach was adopted as the standard for the comparison of gastrin preparations. In cases of very low concentration the gastric fistula stomach gave important supplemental information.

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3. Distribution of gastrin in the tissues

(a) Stomach mucosa

Pylorus. There are twenty-six experiments run with injections of pyloric gastrin into Pawlow animals. The protocol of Dog XI previously given is quite typical of the response. The quantities, the hour following the injection, ranged from 5 to 9 cc. In one case 18.0 cc. was obtained. The free acidities varied between 0.35 and 0.47 per cent, with peptic activity of 16 and 25 mm. of albumin. The response of the gastric fistula dogs is shown in Table I. There were ten experiments with acidities and peptic activities corresponding well to those of Pawlow dogs. The quantities of course were much larger.

Fundus. These preparations showed no appreciable difference in activity from those of the pylorus. If anything, they may have been a trifle more active. The experiments comprised fourteen on Pawlow stomachs and one on a gastric fistula animal (see preceding protocol of Dog IV). The following protocol on Dog III shows the response.

Dog III. Pawlow stomach. May 12

9.	Dressed			
10	1.0 cc.	Free acid 0.200	Total acid 0.273	
10.24	Injected 1 cc. Fundus gastrin			
10.54	6.7 cc.	Free acid 0.373	Total acid 0.419	Peptic act. 28.09
11.24	3.1 cc.	0.501	0.547	12.25
11.54	0.5 cc.	0.474	0.547	
12.24	0.5 cc.	0.182	0.364	
1.24	1.0 cc.	0.082	0.145	

Cardia. The cardiac preparations were a trifle less active than those from the other portions of the stomach. However, the difference in activity was so small that it might easily be attributed to slight variations in the method of preparation. In all, there were six experiments upon Pawlow, and ten upon gastric fistula stomachs. The attached protocol is from a Pawlow dog.

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Dog III. Pawlow stomach. May 8

8	Dressed			
9	1.0 cc.	Free acid 0.228	Total acid 0.282	
10	1.5 cc.	0.027	0.082	
10.30	Injected 1.0 cc. cardiac gastrin			
11	3.6 cc.	Free acid 0.218	Total acid 0.264	Peptic act. 17.64
11.30	1.5 cc.	0.355	0.419	
1.30	2.3 cc.	0.109	0.200	

It is evident from these experiments that gastrin is uniformly distributed throughout the stomach mucosa, with possibly higher concentrations in the fundus.

(b) Duodenal mucosa

Since secretin has been shown to exist chiefly in the duodenum, it was of interest to us to know accurately the gastrin content, in view of a possible relationship of the two bodies. The first preparation showed a variable activity, which was in no sense comparable to that found in the stomach.

Fearing that some activity had been lost by oxidation in the course of preparation, a second sample was rushed through under exact technique, but it showed no greater activity than the first product. A summary of the experiments run is furnished in the Table below.

TABLE II

Experiments on duodenal mucosa

NO. OF ANIMAL	TYPE OF STOMACH	TOTAL NO. OF EXPS.	SLIGHTLY POSITIVE	FAIRLY POSITIVE	STRONGLY POSITIVE	NEGATIVE
I.....	Pawlow, double vagotomy	7	3	3	0	1
III.....	Pawlow stomach	7	2	5	0	0
XI.....	Pawlow stomach	2	0	2	0	0
VII.....	Gastric fistula	5	1	0	4	0
X.....	Gastric fistula	1	1	0	0	0

Slightly positive.—Slight rise in acid without an appreciable increase in quantity of juice.

Fairly positive.—An unmistakable increase in acid and quantity.

A protocol of Dog III which shows the best secretion by any of the Pawlow animals is appended ("fairly positive").

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Dog III. Pawlow Dog. June 30

12.40	Dressed			
1.40	1.0 cc.	Free acid 0.00	Total acid 0.027	
2.40	0.5 cc.			
2.45	Injected 1 cc. duodenal gastrin			
3.45	3.0 cc.	Free acid 0.109	Total acid 0.136	Peptic act. 24.01
4.45	1.5 cc.	0.145	0.218	
5.45	1.2 cc.	0.000	0.063	

While there is a distinct increase in the quantity, yet the acidity rises only slightly. It is not at all of the same order as that following the injection of the stomach preparations.

Below is given a protocol from a gastric fistula dog showing a very pronounced response. It is included for two reasons: first, it will help us in determining the relative concentrations in the duodenum as compared with the oesophagus; second, it is of value in showing how susceptible this type of stomach may be to small stimuli, and therefore, its unreliability as a standard of assay.

Dog VII. Gastric fistula. June 27

10.50	Dressed			
11.50	3.0 cc.	Free acid 0.018	Total acid 0.063	Peptic act. 68.89
12.35	1.0 cc.	Duodenal gastrin		
1.05	12.3 cc.	Free acid 0.337	Total acid 0.355	Peptic act. 33.64
1.35	11.0 cc.	0.419	0.464	21.16
2.35	4.0 cc.	0.109	0.209	
3.35	3.0 cc.	0.000	0.054	132.25

We may conclude that the duodenal preparations give fairly positive gastrin stimulation. The possibility that this is not due to gastrin will be considered later.

(c) Oesophageal mucosa

A tabulation of the experiments run on the oesophageal mucosa will facilitate a comparison with those of the duodenum.

TABLE III

Experiments on the oesophageal mucosa

NO. OF ANIMAL	TYPE OF STOMACH	NO. OF EXPS.	SLIGHTLY POSITIVE	FAIRLY POSITIVE	STRONGLY POSITIVE	NEGATIVE
I.....	Pawlow stomach double vagotomy	2	0	0	0	2
III.....	Pawlow stomach	6	2	0	0	4
VII.....	Gastric fistula	3	0	2	1	0
VIII.....	Gastric fistula	3	0	1	1	1

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A protocol of the best response obtained from a Pawlow dog is given. Such a response as this is labelled "slightly positive," chiefly because of the very slight effect on the total acidity, and because the gastric fistula animals have confirmed this activity by much stronger responses. This experiment should be compared with the experiment on the duodenal extract on the same animal.

Dog III. Pawlow dog. July 20

10.30	Dressed			
11.30	2.0 cc.	Free acid 0.000	Total acid 0.054	Peptic act. 10.89
11.43	Injected 1 cc. oesophageal gastrin			
12.43	2.1 cc.	Free acid 0.000	Total acid 0.072	19.36
1.43	1.7 cc.	0.000	0.045	
1.43	Injected 1 cc. oesophageal gastrin			
2.43	2.3 cc.	Free acid 0.000	Total acid 0.072	32.49
3.43	2.7 cc.	0.000	0.054	14.44

An experiment on Dog VII is introduced for comparison with the stimulating effects of the duodenal mucosa on the same animal.

Dog VII. Gastric fistula. July 10

12.20	Dressed			
1.20	0.7 cc.			
2.20	3.5 cc.	Free acid 0.000	Total acid 0.027	Peptic act. 2.25
2.30	Injected 1 cc. oesophageal gastrin			
3.30	2.5 cc.	Free acid 0.000	Total acid 0.054	Peptic act. 3.61
4.30	6.0 cc.	0.000	0.127	
4.35	Injected 1 cc. oesophageal gastrin			
5.35	20.0 cc.	Free acid 0.355	Total acid 0.401	Peptic act. 4.84
6.35	6.0 cc.	0.264	0.319	27.04

If Tables II and III are compared, it will be seen that so far as the Pawlow animals are concerned, the duodenum contains a much higher concentration of gastrin. In truth with no further evidence, one would say that it was absent in the oesophagus. If the two experiments on Dog VII are compared, it will be seen that a second dose of oesophageal gastrin was necessary to provoke a response equal to one obtained by one dose of duodenal mucosa. Gastrin therefore appears to be present in very small quantities in the oesophageal mucosa.

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(d) Pancreas

Five experiments were made on four different Pawlow dogs. Of these only one showed evidence of secretion, in which case the quantity of juice was not altered, but the total acidity rose from 0.291 the hour preceding the injection, to 0.364 the second hour after the injection. In the case of the gastric fistula dogs, two experiments on Dogs X and XIII were entirely negative; four others on Dogs VII, VIII and IX were labelled as negative or suspicious. In the latter cases there was a slight increase in volume, associated with very evident quantities of bile. One sample macroscopically appeared to be pure bile. Upon such data we feel justified in concluding that the pancreas furnished no gastrin.

(e) Submaxillary gland

Six experiments on four Pawlow dogs were all negative. Five experiments on three gastric fistula animals were negative, one on a gastric fistula dog with double vagotomy and splanchnics cut was suspicious. The submaxillary gland does not furnish gastrin.

(f) Striated muscle

In three experiments run on two Pawlow dogs, no secretion was obtained. In two experiments on a Pawlow dog (doubly vagotomized) a slight effect is shown as follows. The hour preceding the injection 1.8 cc. of juice was secreted, with free acid 0.246, total acid 0.319. The hour following 2.5 cc. juice, free acid 0.282, total acid 0.328 were obtained. An interpretation of this latter result is not quite clear, since it involves a question of mechanism, a subject which is under investigation. It is sufficient to say that if gastrin be present, it must be in exceedingly minute quantities, otherwise it would have been shown by the normal Pawlow dogs.

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(g) Smooth muscle

The muscle from the stomach wall was used for this preparation. Four experiments on four different Pawlow dogs proved negative. One of these negative experiments was made on the animal with double vagotomy, which had previously shown a slight reaction to the striated muscle.

(h) Brain tissue

These experiments are of unusual interest, because they have given us a juice which appears to be other than a true secretion. The tests comprised four experiments on four Pawlow dogs. Two were negative, and two gave a marked increase in volume with no rise in acidity, but with a decrease in pepsin. The protocol attached is illustrative.

Dog XII. Pawlow stomach. August 28

10.35	Dressed			
11.35	1.4 cc.			
12.35	0.6 cc.	Free acid 0.000	Total acid 0.063	Peptic act. 151.29
12.55	Injected brain gastrin			
1.55	3.0 cc.	Free acid 0.000	Total acid 0.018	42.25
2.55	1.6 cc.	0.000	0.018	

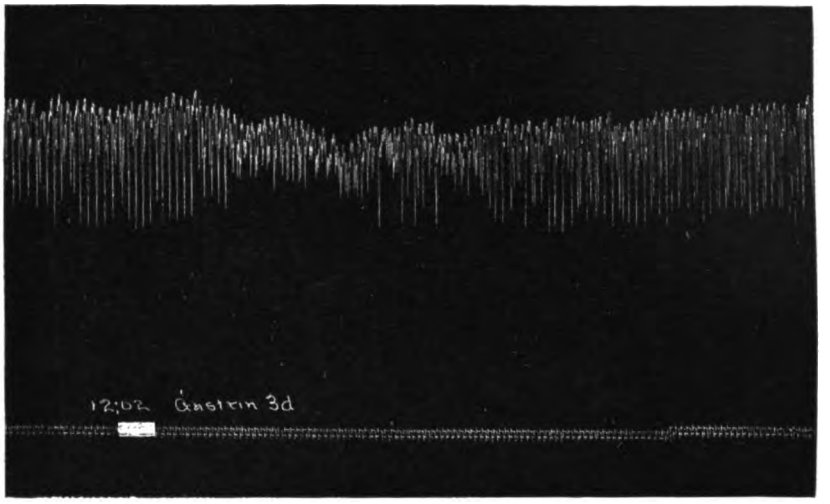
This is certainly not a typical gastrin response.

4. Effect of gastrin preparations on blood pressure

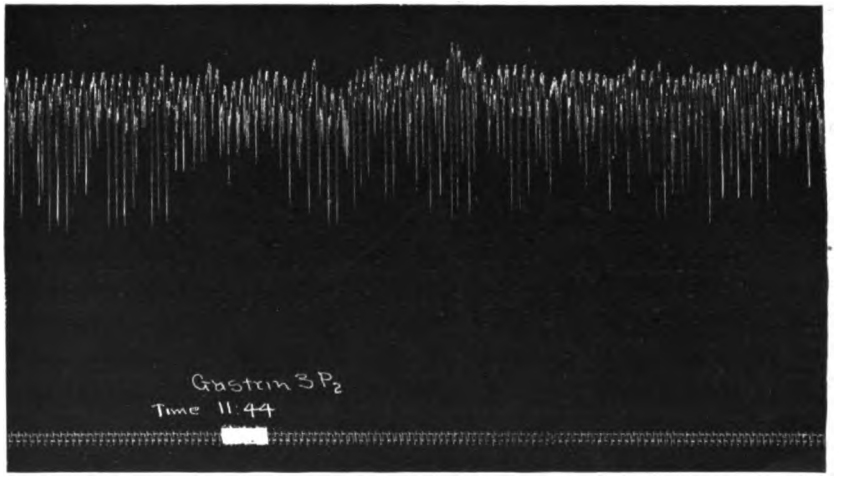
It will be recalled that, in making these gastrin preparations, an effort was made at the last to remove the "vasodilatins" with hot absolute alcohol. In view of the oft-repeated contention of Popielski that all of the effects of injections of this class of substances were to be attributed to vasodilation, it became important to know just what were the blood pressure changes under the condition of the experiment. The method of Brooks,[18] of taking the blood pressure without an anaesthetic was adopted. The cannula was introduced into the carotid under ether, from which the animal was allowed to recover completely (usually

[18] Clyde Brooks: Heart, 1910–11, ii, 5.

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Gastrin 3d: An active preparation from the cardiac part of the gastric mucosa. Time tracing shows seconds.



Gastrin 3P2: The same brain preparation as was used in the previous protocol. Time tracing shows seconds.

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one hour is sufficient) before the manometer was attached. The tracing shown below demonstrates that we still have depressor substances present in the preparation. The fall is transient, lasting four to five minutes, but reference to the first protocol (Dog IV) shows that the secretion is at its height thirty minutes after injection. The points of maximum blood pressure depression and maximum secretion do not coincide. It is to be noted that the active gastrin preparation lowers the blood pressure more than the brain preparation. The intravenous injection of our preparations like the secretin preparations of Bayliss and Starling cause a marked temporary fall in blood pressure.

5. Effect of anaesthetics on gastrin stimulation

Following Edkins' method of experimenting, attempts were made in decerebrated cats and etherized dogs, using both intravenous and intramuscular injections, to demonstrate the activity of our preparations. No measurable secretion could be obtained, and we feel that any conclusions based on such a method are open to serious objections.

E. THEORETICAL CONSIDERATIONS AND CONCLUSIONS

1. Nature of the mechanism

A review of the data given above shows that we may consider a one to two hours' secretion of a greater volume of gastric juice together with a higher concentration of acid and an increase in the total output of pepsin as a typical response to an intramuscular injection of gastrin. These results mean that the glands have been stimulated to do work, and this work is not a small, measurable amount, but a quantity of striking magnitude if expressed in physical units.

We feel that Popielski's contention that simple "vasodilatin" action is responsible for this increased secretion is not supported by his, nor by our observations. In the first place, he has not by quantitative methods shown the character of the

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responses in different digestive glands after the injection of even crude tissue extracts. Second, although we have not been able to obtain our preparations free from depressor substances by the method recommended by Bayliss and Starling, we have not observed the maximum secretion to take place at the time of maximum fall in blood pressure, but some twenty-five to thirty minutes after the return to normal pressure. Third, we have never observed salivation. Fourth, different tissue extracts exert different effects; the gastric mucosa is most active, the duodenal less so, and the oesophageal only faintly; others are inactive, and brain extract prepared in the same way causes an abnormal secretion, free from acid, lower in pepsin, but greater in volume. Fifth, in two attempts to demonstrate pancreatic juice secretion by means of gastrin, we obtained negative results. Sixth, the indications are that the active ingredient in our gastrin preparations is more stable than the pancreatic secretin of Bayliss and Starling.

As stated above, our preparations, like all the active secretin preparations of Bayliss and Starling or of Dixon and Hamill, were by no means free from blood pressure lowering action, but this does not necessarily call for a general secretion of digestive juices, nor does it follow that the active principle itself, if a true specific secretagogue, causes this fall in blood pressure. As we have a rapid return to normal general blood pressure, but a prolonged secretion, we may consider the action as due possibly to a marked preliminary splanchnic or local vasodilatation followed by a compensatory peripheral constriction; or we may have both a splanchnic vasodilatation as well as a specific stimulation of the gastric glands directly or of the intrinsic nerves of the stomach walls, the actions being due to the same or different substances. Certainly a local vasodilatation is to be expected, and after the primary stimulation of the cells may be an important contributary factor to activity; we have however no experimental data on this point. To be sure our negative results as to other secretions may be due to a more irritable state of the gastric circulation to these general vasodilatins; if we admit this we at once introduce the question of differential

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specificity. Again, our negative results with other extracts may in part be due to the lower concentrations of the vasodilatins in these tissues, but we cannot entirely explain the negative results thereby, nor can we so simply explain the abnormal results with brain extract. Two other alternatives occur to us as to these negative results. They may be due to a less complete extraction of the active substances from these tissues since in the preparation of gastrin solutions from gastric mucosa we always have a very much more complete disintegration of the tissue. This disintegration is no doubt due to the digestive action of pepsin in spite of the rapid heating to 90° C. The other alternative is that the peptic digestion of proteins may yield gastrin or substances of similar nature. The abnormal response after brain gastrin may be due to a local or general vasodilatation together with a toxic action on the cells or together with the absence of the specific stimulant for gastric secretion. We admit that in the present state of our knowledge all explanations proposed are necessarily decidedly speculative. We are investigating various phases of these questions, and hope that the purification of the active substance will do much toward cleaning up the same.

2. Distribution of gastrin bodies

It will be well at this point to summarize the findings of others as to the distribution of gastrin activity, in so far as they bear on our results.

Edkins found the activity of the stomach preparations confined to the pylorus. It is sufficient to say that all of his experiments were done under an anaesthetic, that his methods of juice collection were not adapted to the studies of the character of the response, and that his preparations contained a maximum of the vasodilatins (Popielski.)

Eisenhardt worked with juice collected from different portions of the stomach, which was reinjected. The fundus juice was not active, hence his findings do not necessarily controvert our results.

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Maydell was unable to find activity in the fundus portion of the stomach. Ehrmann, Tomaszewski, and Emsmann agree with us as to the activity of the fundus portion of the stomach.

Emsmann found the pancreatic extracts active, but not of the same order as the pyloric ones. His experiments were not strictly quantitative, hence his results are border line cases, and their interpretation hinges on the question of what constitutes a positive response.

3. Relation of gastrin to pancreatic secretin

As to the possible relationship of gastrin and secretin, we have no definite experimental data to offer. Maydell and Bayliss and Starling have not found that pancreatic secretin stimulates the gastric juice. The studies of Dixon and Hamill, Bayliss and Starling and others, have shown secretin to be a relatively unstable body. On the other hand, gastrin is quite stable as shown by our experience and that of Tomaszewski, who was able to heat it to 116° for an hour. Until our product is isolated and its effects on pancreatic secretion are established, we cannot state that the duodenal preparations are stimulating the gastric mechanism primarily, or secondarily through the presence of pancreatic juice in the duodenum. The latent period and the character of the response at present incline us to the view that the duodenal extracts are stimulating the stomach primarily and that they merely contain a smaller concentration of the active substance.

4. Specificity of gastrin

The differential distribution of gastrin in the gastro-intestinal tract, and the negative results with tissues of widely varying chemical constitution suggest that it is a specific substance rather than a combination of protein split products.

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CONCLUSIONS

1. Evaporation of an acid extract from various tissues leaves a residue, difficultly soluble in 95 to 98 per cent alcohol, which manifests varying degrees of gastrin activity.

2. The gastrin is uniformly distributed throughout the stomach mucosa, is found in much smaller concentrations in the duodenum, and its presence can just be demonstrated in the oesophagus. Preparations of pancreas, submaxillary gland, smooth muscle, and striated muscle are negative.

3. The brain gastrin furnishes an abnormal type of secretion.

4. Pawlow stomachs have been found more satisfactory than gastric fistula stomachs for assaying the preparations. Injections were made intramuscularly.

5. Gastrin in intramuscular doses of 1 cc. (corresponding to 4 to 5 grams of fresh tissue) cause a fall in blood pressure lasting over four to five minutes, a secretion lasting over one and one-half hours, with a maximum between thirty and forty-five minutes following the injection.

6. It is our belief that gastrin causes a true gastric secretion rather than a simple vasodilator response, that it is of a different chemical nature from pancreatic secretin, and that it is a specific substance.

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RESEARCHES ON THE EXCHANGE OF ENERGY IN LIVE ANIMAL TISSUES

I. Micro-Calorimetry Applied to Animal Tissues

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Thus far, we believe, the intensity of movement of energy in live animal tissues separated from the organism has been measured by the proportion of exchange of gaseous matter occurring therein. The heat generated by them is measured by respiratory calorimetry. We shall later on discuss the value of the process as applied to this individual case. In this paper we shall attempt to take up the manner in which one may, with ease and accuracy, measure the heat developed by animal tissues separated from the organism, and, at the same time, we shall endeavor to determine the proportion of their exchange of gaseous matter. In doing this, it is our purpose: (1) To ascertain the value of the heat generated by each different animal tissue; (2) To compare the results of indirect respiratory calorimetry, with those obtained by direct means.

MICRO-CALORIMETRY

If a vessel, or receptacle, of perfectly non-conducting material were available, the problem of micro-calorimetry would be reduced to measuring the increase in temperature (x) of the vessel and its contents, during the experiment, and multiplying this increase in temperature by the water-value (V) of the calorimeter and the substance to be investigated. The product (Vx) would exactly represent the amount of heat sought for. Unfortunately, such vessels, or receptacles, do not exist and even the best available allow quite perceptible amounts of heat

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to escape. In order to obtain the total value of heat generated it is, therefore, necessary to ascertain such loss, and to add it to the expression (Vx) representing the heat retained by the calorimeter. Let us take any vessel or receptacle; the amount of heat which it allows to escape within a specified time will depend on the nature and thickness of its walls, on the condition of its surface, the environment in which it is located, and the difference (T–T_o) between the temperature of the vessel (T) and the outside temperature (T_o); therefore, in the case of an identical vessel, placed in the same environment, the amount of heat lost will depend only on (T–T_o). Experience shows that within the limits of the temperatures used for physiological researches there is a perfect proportion between the amount of heat lost by conduction (Q) and the difference (T–T_o) between the temperature of the vessel and the outside temperature. Therefore, we find,

$$\frac{\zeta}{(T - T_0)} = \text{constant}$$

It is this peculiar feature of the calorimetric vessels, which makes it possible to ascertain the heat lost by conduction. This constant was adopted, after proper trial, by Rubner (1) and Hill (2) the authors of the only two methods of micro-calorimetry which, to the best of our knowledge, exist in physiology. Rubner adopted a constant (K) representing the amount of heat that a calorimetric vessel allows to escape during one hour (T–T_o) being equal to 1°C. Hill adopted a constant (k) representing loss of temperature, the connection between same and the constant K (loss of heat) being represented by the relation:

$$k = \frac{K}{\text{water-value of the calorimeter and its contents}}$$

Let us now suppose that during an experiment the average value of (T–T_o) is equal to (m) degrees. The heat loss will be equal to (mK). In actual practice, however, how can we determine the average value of (T–T_o) during an experiment? According to Rubner and Hill, it will not be possible to establish this average value of (T–T_o) whenever the outside temperature (T_o)

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should happen to oscillate arbitrarily. It is, therefore, absolutely necessary to annul, or counter-balance, the variations of the outside temperature. Rubner succeeded in obtaining this by placing the calorimetric vessel in an incubator the temperature of which was maintained absolutely stable by a complicated and expensive process.

Hill preferred to annul, or counterbalance the variations of (T_o) by connecting the calorimeter used in the experiment to another controlling vessel, in accordance with the differential method. He then demonstrated that, if k and k', coefficients of loss of temperature of these vessels, are equal to each other, it is possible to ascertain the total amount of heat generated, given the value of (T–T') during the experiment, the difference between the respective temperature of the two calorimetric vessels being obtained directly by the mere reading of a galvanometer connected to two thermo-electric solders, placed respectively one in each of the calorimetric vessels.

Rubner's method requires a difficult installation in order to be carried out in practice, and presents no advantages of precision or convenience over that of Hill. Hill's method, according to the author, has the great advantage of not requiring continual readings of the difference of temperature (T–T'), that such readings need be made only every hour or even every two hours, during the longer tests. This fact is a consequence of the regularity with which (T–T') oscillates, all irregular variations of the outside temperature having been annulled as we have seen. This advantage is not, however, peculiar to the method, inasmuch as (T–T') depends also of the manner in which the heat is developed within the calorimeter. It suffices, for instance, that heat be generated in an irregular manner, for (T–T') also to oscillate irregularly, thereby rendering absolutely necessary the continual reading of the differences of temperature (T–T').

Furthermore k and k' always differ in practice by a perceptible amount which varies at each experiment. To this is perhaps due the fact that his method shows in trial tests on an average, errors of 4.8 per cent and 5.3 per cent. If we take the extreme errors (−7.7 per cent and 9 per cent) of a series,

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we find, on comparing the two experiments, that the error may amount to 16.7 per cent. If we take the extreme differences of the series which the author states was carried out with more care (series of 3 tests, average error = 3.2 per cent, extreme errors = −3.9 per cent and 3.8 per cent) we shall find 7.7 per cent. The amplitude of these errors is excessive for the problem which we purpose solving. Moreover, Hill's method requires the use of the thermo-electric process to obtain the differences of temperature (T–T') and, the use of a good galvanometer, which cannot always be found in all laboratories.

We shall now proceed to describe the method used by us and which, although relatively elaborate, is as precise as may be desired, and requires so simple an outfit as to be within the reach of any investigator. The solutions of Rubner and Hill are, as we have just seen, but the direct consequence of their belief that it is only possible to calculate the losses in heat by conduction, or else when the outside temperature does not oscillate or, again, when such oscillations are annulled (counter-balanced) by the differential method. Their belief does not, however, strictly correspond to the actual facts. Let us imagine a calorimetric vessel having in its interior one of the solders of a thermo-electric battery, the other solder being placed outside the calorimeter. The readings of the galvanometer will show the difference (T–T_o) between the temperature of the calorimeter (T) and that of the outside (T_o). Based on the frequent readings taken, let us make a record of the differences (T–T_o) on chart paper, this curve showing the time at the axis of x and the differences (T–T_o) as ordinates. As the calorimetric vessel admits of a constant (K) of loss in heat, it is necessary to

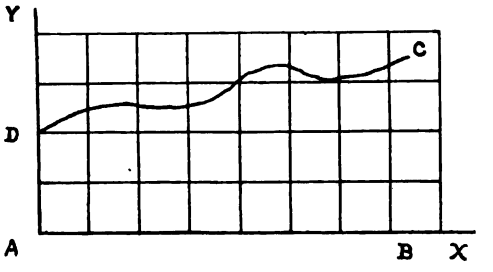


Fig. 1

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find out, in order to ascertain the heat lost by conduction, the average difference of $(T-T_o)$ for the length of time during which the experiment was conducted. The problem, therefore, is reduced (as shown in fig. 1) to determine the average ordinate of the curve $(T-T_o)$.

It is evident that the area of the surface $ABCDA$ is equal to the base AB multiplied by the average height. AB being known, the area $ABCDA$ can easily be determined (as closely as desired) by the usual different methods of squaring. The unknown term, the average distance from curve $(T-T_o)$ to base AB, is, therefore, equal to the area of the surface $ABCDA$ divided by AB.

In other words, $ABCDA = s$ and $AB = t$, or the duration of the test.

We therefore have; Average value of $(T-T_o) = \frac{s}{t}$.

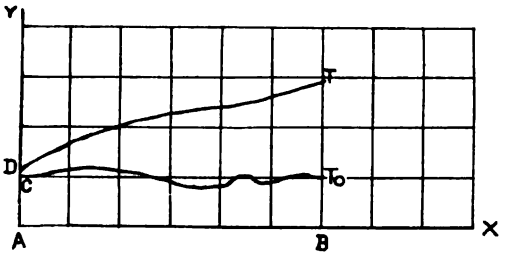


Fig. 2

Let us now suppose that we have no galvanometer to obtain directly the difference $(T-T_o)$. It will then suffice to place a thermometer in the calorimeter and with another thermometer follow the oscillations in the outside temperature. By charting these readings to two coördinate axes we shall obtain the two curves shown in the figure 2. By similar reasoning, it will be seen that the average difference of temperature $(T-T_o)$ is equal to the surface CT_oTDC divided by its projection over the axis of x (that is by AB) time of duration of the experiment. These are the principles on which we have sought to ascertain the heat generated, and which we may subdivide into two parts:

The first, which is retained by the calorimeter, is equal to the water-value (V) of the calorimeter and of the matter which is being investigated, multiplied by the differences of temperatures inside the calorimeter, from the time the experiment is started

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until its conclusion (= x); the second part that which is lost by conduction, is equal, as we have seen, to $K\frac{s}{t}$.

The total amount of heat (Q) is therefore represented by the expression

$$Q = Vx + K\frac{s}{t}$$

A FEW PARTICULARS OF OUR METHOD OF MICRO-CALORIMETRY

Our calorimeter is, like Hill's, the Dewar flask, i.e., a vessel or receptacle, with silver plated double glass walls, separated by vacuum, such as the bottle generally and commercially known as "Thermos." Its mouth or opening is closed by a rubber stopper. This stopper has the following devices: (1) A very precise and sensitive thermometer, previously verified, and capable of recording up to one hundredth of a degree centigrade. The mercury reservoir of this thermometer reaches down in the vessel, almost touching the bottom thereof; (2) Two fine tubes, to which are adapted rubber tubes which extend outside the vessel, the extremities of these rubber tubes being closed and tied with thread during the experiments. It is to be noted that the glass tubes proper fit exactly into the stopper, and do not project beyond same, and they are used for the purpose of collecting the gases from the calorimeter, for later analysis.

To determine the water-value of the calorimetric vessel, the following method is adopted:

A certain quantity (P) of water is placed inside the vessel, and after a certain time has been allowed to lapse, the temperature (T) of the vessel and of the water is taken; a further amount of water (p) is then added of the temperature (t) and a reading is taken of the final temperature (t'). Let (x) be the water-value of the calorimetric vessel; (t') is equal to:

$$t' = \frac{xT + PT + pt}{x + P + p} \therefore \quad x = \frac{t'(P + p) - (PT + pt)}{T - t'}$$

By repeating this procedure several times, adding water now warmer than that in the calorimeter, now colder, one is able to

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determine very closely the constant of the apparatus. It is a mere matter of care and practice.

Constant K representing the loss in heat, is determined in the following manner: In the calorimeter, the water-value of which is already known, a weight (P) of water is poured, at a temperature (T), and the apparatus and its contents are then allowed to cool. Frequent readings of the inside temperature are taken as the process of cooling progresses, and, at the same time, record is kept of the outside temperature. These two series of readings are transcribed to chart paper, on which reference is likewise made of the time. The average value of $(T-T_0)$ is determined by the methods described above.

Let us suppose that the vessel became cooler by x degrees. The amount of heat that is lost by conduction, for 1° of difference, is equal to

$$\frac{\text{(water-value of the calorim. and its contents)} \times x}{\text{average value of } (T-T_0)}$$

This result is reduced to 1 hour by a simple proportion. The tests we made, in connection with the proportion of heat lost in relation to the difference in temperature $(T-T_0)$, namely, the verification of the existence of a constant K, consisted in allowing the calorimeter to cool, a given or specific amount of water having been placed in it, due record being made at the same time of the variations in the outside temperature, so as to ascertain the average value of $(T-T_0)$. These experiments, or tests, although carried out according to different technical procedures, confirm those made by Rubner and Hill, with but one restriction: the constants k or K vary slightly in accordance with the environment (air or water, for instance) in which the calorimeter is placed. It is, therefore, evidently necessary always to place the calorimeter in the same environment, whenever a precise experiment is desired.

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PREPARATION OF THE ANIMAL TISSUES FOR EXPERIMENTS OF MICRO-CALORIMETRY

We have, in a measure adopted the technical process of Battelli-Stern (3), which, however, we have endeavored to improve in order that it might the better answer our purpose. In the experiments carried out, we have always made use of tissues taken from a dog immediately after its death by bleeding. The organ (the liver, for instance), is quickly cut into pieces, and finely ground thereafter in Lattapie's apparatus. The pulp thus obtained is placed in a capsule of a specific tare, so as to attain 50 grams. This pulp is then suspended in a fluid which, for the sake of convenience we shall call artificial blood, and which is obtained in the following manner: 125 grams of blood of the dog whose organ is to be experimented upon are placed into an Erlenmayer vessel or receptacle, containing small glass balls (pearls); it is then shaken, filtered through a cloth to separate the fibrin, centrifugated to separate the red globules; the serum is then decanted. The original volume is reëstablished by the addition of a simplified formula of Locke's fluid as follows:

NaCL.....	9.0 grs.	$CaCl_2$.....	0.20 centgrs.
KCL.....	0.20 centgrs.	Water.....	1000.0 grs.

The whole is again centrifugated, and the preceding operation is repeated retaining the previous volume of 125 grams. Thus, one will have obtained a suspension of red globules in Locke's simplified fluid. The purport of all this is merely to eliminate the blood serum and, with it, the salts capable of collecting carbonic acids. Indeed, when this precaution is not taken, the proportion of CO_2 and the respiratory quotient of Pflüger are likely to vary much in similar experiments, while, if the course we have outlined is followed, this will be avoided. For the same reason, we simplified Locke's fluid, eliminating the bicarbonata. The respiration of the various tissues occurs, under the circumstances with great vigor and to the satisfaction of the investigator.

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HOW TO CONDUCT THE EXPERIMENTS

The tissues suspended in the artificial blood is placed in a glass jar of about 1 liter capacity, which is then stoppered and submerged in the water of an incubator the temperature of which should be more or less the same as that at which the experiment will be made. Our incubator consists of a zinc tank with about 60 liters of water. The temperature is maintained by gas burners connected to an ordinary regulator. In this tank should also be placed the calorimeter unstoppered, as well as its rubber stopper in which, as already explained above, a thermometer is inserted. Every now and then the water which enters the Thermos (calorimeter) is emptied out and the receptacle is allowed to replenish. By this procedure, and exercising particular care, the same temperature is obtained for the calorimeter, its stopper and the tissue on which the experiment is to be made. This method also has the advantage of saturating the red globules of the artificial blood with oxygen. The calorimeter is emptied out the last time, without removing it from the incubator, the emulsion of tissues is poured into it and the receptacle is closed with the stopper fitted with the thermometer. The calorimeter is then attached to a shaker consisting of a wooden board plunged into the water of the incubator. This board is connected, on its other side by means of a suitable device, to an electric motor which will cause it to move back and forth. Thus the double result is obtained: (1) shaking in a thorough and uniform manner the emulsion in contact with the air obtained in the calorimeter, which facilitates its respiratory exchange, and (2) rendering homogeneous the temperature of the water in the incubator, which is most convenient for ascertaining the exact outside temperature (T_o). A good thermometer, similar in all respects to that which is fitted to the stopper of the calorimeter, is dipped into the water of the incubator. When the experiment is concluded, the calorimeter is immediately removed from the incubator and the gases, which are necessary for subsequent analysis, are collected from the glass tubes fitted into the stopper of the calorimeter, as already explained.

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REMARKS

The calculation of the total heat generated is represented by the formula

$$\zeta = Vx + K\frac{s}{t}$$

For the first part, thereof, it is always very hard to ascertain the value of the specific heat of the material under examination and it is therefore generally considered as equal to that of water. This may, however, mean a very perceptible error. Inasmuch as the second part is not subject to the same mistake, and is obtained with much more accuracy it is often preferable to increase considerably the difference $(T-T_o)$ between the temperature of the calorimeter and that of the outside environment, by allowing the latter to become cooler. Under the circumstances, the expression $K\frac{s}{t}$ increases at the expense of the expression Vx which may be annulled or rendered negative. The oscillations of the temperature of the incubator, which is equipped with an ordinary regulator are very slow and this makes it easier for the investigator. As a rule, our experiments are conducted with the calorimeter completely submerged in the water of the incubator; the incubator, however, is not really indispensable, and the experiments may be conducted in the open air.

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